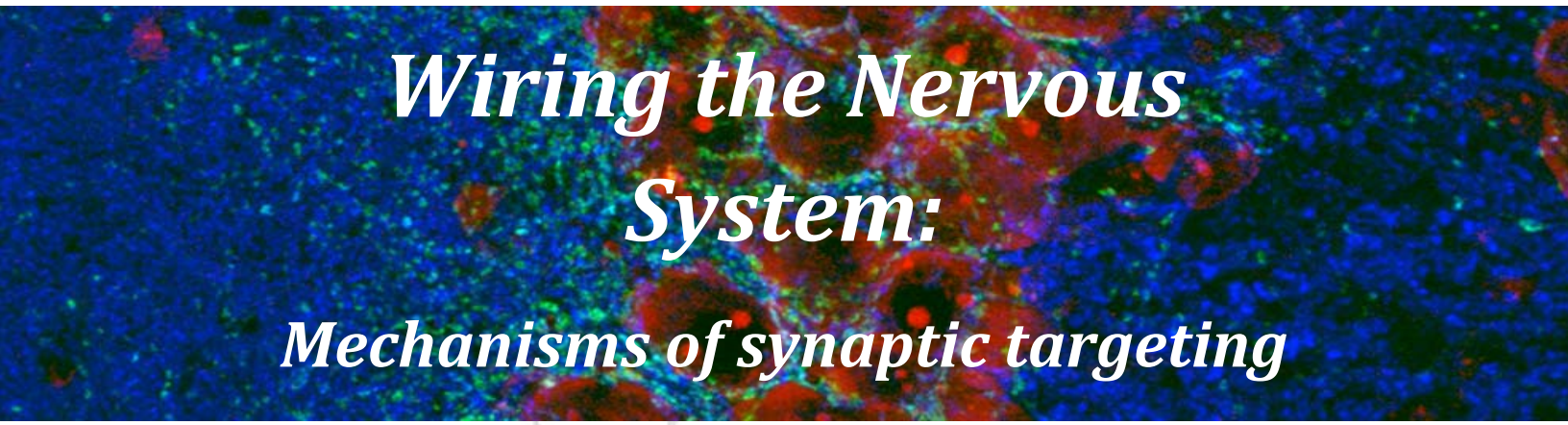

Wiring the Nervous System:

Mechanisms of synaptic targeting

The Central Virginia Chapter of the Society for Neuroscience Annual Symposium

Virginia Commonwealth University

Richmond, VA

MARCH 16TH 2012

8:00 AM	REGISTRATION OPENS
9:00 AM	OPENING REMARKS
9:15 AM	DR. SAMUEL PFAFF'S TALK ENTITLED *"GENETIC CHARACTERIZATION OF SPINAL NEURON CONNECTIVITY"*
10:15 AM	COFFEE BREAK
10:30 AM	DR. ALEX KOLODKIN'S TALK ENTITLED ***"****MOLECULAR MECHANISMS UNDERLYING THE ESTABLISHMENT OF NEURAL" CONNECTIVITY*
11:30 AM	POSTER SESSION AND LUNCH (IN KONTOS MEDICAL SCIENCES BUILDING, ROOM 104/105)
2:00 PM	DR. CAROL MASON'S TALK ENTITLED ***"****PATTERNING THE VISUAL PATHWAYS FROM EYE TO BRAIN"*
3:00 PM	DR. JOSHUA R. SANES' TALK ENTITLED ***"****ASSEMBLING NEURAL CIRCUITS IN THE VISUAL SYSTEM"*
4:00 PM	CLOSING REMARKS
4:30 PM	RECEPTION

PLENARY LECTURES

SAMUEL PFAFF:

"Genetic characterization of spinal neuron connectivity"

Professor in the Gene Expression Laboratory at the Salk Institute. In addition to being a Howard Hughes Investigator, Dr. Pfaff was a Pew Scholar and McKnight Scholar and received the Basil O'Connor Award.

ALEX KOLODKIN:

"*Molecular mechanisms underlying the establishment of neural" connectivity*

Professor of Neuroscience at Johns Hopkins University School of Medicine. Dr. Kolodkin is not only a Howard Hughes Investigator, but has received the McKnight Neuroscience investigator Award, the Jacob Javits Neuroscience Investigator Award, and was a Searle Scholar.

CAROL MASON:

"*Patterning the visual pathways from eye to brain"*

Professor of Pathology and Cell Biology, Neuroscience and Ophthalmic Science at Columbia University. Among other awards, Dr. Mason has received an NIH Research Career Development Award, the Jacob Javits Neuroscience Investigator Award and is a Fellow of the American Association for the Advancement of Science.

JOSHUA R. SANES:

"*Assembling neural circuits in the visual system"*

Paul J. Finnegan Family Director of the Center for Brain Science and Professor of Molecular and Cellular Biology at Harvard University. Among his many awards, Dr. Sanes is a member of the US National Academy of Sciences, a Fellow of the American Association for the Advancement of Science, a recipient of the Alden Spencer Award of Columbia University and a recipient of the Jacob Javits Neuroscience Investigator Award.

POSTER ABSTRACTS

Posters will be displayed in rooms 104 and 105 of the Kontos Medical Sciences Building.

Abstract Number	Presenter	Presenter Type	School
1	L.Sullivan	*Undergraduate*	George Mason University
2	M.Rubaharan	*Undergraduate*	George Mason University
3	S.Iyer	*Graduate student*	George Mason University
4	C.Waggener	*Graduate student*	Virginia Commonwealth University
5	M.McDonough	*Undergraduate*	College of William and Mary
6	B.Rabe	*Undergraduate*	College of William and Mary
7	W.Herbst	*Undergraduate*	College of William and Mary
8	J.Chan	*Graduate student*	Virginia Commonwealth University
9	A.Doperalski	*Postdoc*	Virginia Commonwealth University
10	T.Taetzsch	*Graduate student*	Virginia Commonwealth University
11	M.Surace	*Postdoc*	Virginia Commonwealth University
12	A.Vestal-Laborde	*Graduate student*	Virginia Commonwealth University
13	K.Edamura	*Graduate student*	University of Virginia
14	H.Herman	*Graduate student*	Virginia Commonwealth University
15	S.Vunck	*Graduate student*	Virginia Commonwealth University
16	J.Chojnacki	*Graduate student*	Virginia Commonwealth University
17	S.Snider	*Graduate student*	Virginia Commonwealth University
18	K.Hardcastle	*Undergraduate*	College of William and Mary
19	S.Haque	*Undergraduate*	College of William and Mary
20	K.Zajo	*Undergraduate*	College of William and Mary
21	B.Costin	*Graduate student*	Virginia Commonwealth University
22	M.O'Brien	*Graduate student*	Virginia Commonwealth University
23	N.Rao	*Graduate student*	University of Virginia
24	B.Sharp	*Graduate student*	University of Virginia
25	K.Mirowska	*Graduate student*	University of Virginia
26	Y.Kim	*Graduate student*	Virginia Commonwealth University
27	E.Solis	*Graduate student*	Virginia Commonwealth University

28	K.Cameron	*Graduate student*	Virginia Commonwealth University
29	L.Binari	*Technician*	University of Virginia
30	J.Eckardt	*Graduate student*	University of Virginia
31	L.Groskaufmanis	*Undergraduate*	University of Virginia
32	T.Huntington	*Undergraduate*	University of Virginia
33	J.Kennett	*Postdoc*	University of Virginia
34	R.Nash	*Undergraduate*	University of Virginia
35	V.Patel	*Undergraduate*	University of Virginia
36	A.Peruri	*Undergraduate*	University of Virginia
37	H.Shim	*Graduate student*	Virginia Commonwealth University
38	L.Kondo	*Graduate student*	Virginia Commonwealth University
39	E.Hawkins	*Graduate student*	Virginia Commonwealth University
40	P.Muldoon	*Graduate student*	Virginia Commonwealth University
41	Y.Hahn	*Graduate student*	Virginia Commonwealth University
42	T.Seabrook	*Graduate student*	Virginia Commonwealth University
43	M.Wallace	*Undergraduate*	James Madison University
44	C.Klotz	*Undergraduate*	James Madison University
45	J.Brooks	*Graduate student*	Virginia Commonwealth University
46	J.Su	*Postdoc*	Virginia Commonwealth University
47	A.Moore	*Undergraduate*	College of William and Mary
48	R.Perez	*Undergraduate*	College of William and Mary
49	J.Wang	*Graduate student*	Virginia Commonwealth University

Abstract 1

CHARACTERIZATION OF DOWNSTREAM EFFECTORS MEDIATING CUT TRANSCRIPTIONAL REGULATION OF CLASS-SPECIFIC DENDRITE MORPHOGENESIS

Luis Sullivan, Eswar P.R. Iyer, Madhu Karamsetty, and Daniel N. Cox

School of Systems BIology, Krasnow Institute for Advanced Study Mail Stop 2A1, George Mason University, Fairfax, VA 22030

Neuronal form dictates function and in a circuitry as complex as the human brain the post-synaptic properties of the neuron are established in large part by dendritic morphology. Transcriptional regulation has emerged as a pivotal mediator of class specific dendrite morphogenesis; however, the downstream effectors of these transcription factors remain largely unknown as are the cellular events that direct morphological change. Recent studies have implicated the Drosophila homeodomain transcription factor Cut and it's vertebrate homolog in mediating dendrite morphogenesis in the peripheral and central nervous systems. To characterize putative transcriptional targets of Cut regulation, a genetic suppressor screen has been performed in which Cut overexpression has been coupled with target gene-specific in vivo RNAi knockdown. Preliminary analyses have identified >400 genes that represent potential direct targets of Cut regulation in Drosophila dendritic arborization (da) neurons. Here we report the discovery of target genes that either suppress or enhance Cut-mediated effects on da neuron dendritic morphology. The molecules uncovered in our screen cover a broad range of biological functions. Collectively, these analyses reveal novel transcriptionally regulated pathways and cell biological processes essential to the specification of class specific dendritic morphologies.

Abstract 2

CHARACTERIZATION OF DAR1 INTERACTING PROTEINS ESSENTIAL FOR DIFFERENTIAL CELLULAR LOCALIZATION AND REGULATION OF CLASS-SPECIFIC DENDRITE DEVELOPMENT

Myurajan Rubaharan, Srividya Chandramouli Iyer, Eswar P.R. Iyer, Daniel N. Cox. Daniel Cox

Krasnow Institute of Advanced Study, George Mason University, 4400 University Drive, Fairfax, Virginia, 22030.

Dendrite morphogenesis represents a critical process in the establishment, maintenance and modulation of neural connectivity that is the basis of a functional nervous system. Dendrites, as the primary sites of synaptic and/or sensory input largely determine the size and range of the neuronal receptive field. A recent study has identified dar1, a Krüppel-like transcription factor, as an essential regulator involved in controlling dendrite development and growth via microtubule modulation. The Drosophila melanogaster peripheral nervous system (PNS) has emerged as an excellent model system for studying molecular mechanisms underlying class specific dendrite development. Dendritic arborization (da) neurons are grouped into four distinct classes (I-IV) based upon increasing orders of dendritic complexity. Interestingly, Dar1 protein localization is primarily nuclear in the morphologically simple class I neurons, whereas in contrast largely cytoplasmic in the highly complex class IV da neurons. This observation led us investigate putative protein-interaction partners of Dar1 that potentially regulate this complexity-dependent differential localization, and the result of perturbing this localization. A targeted mutant screen for nineteen genes identified as direct interactors of Dar1 by protein-protein interaction studies recovered genes responsible for its differential localization in class IV da neurons. This study sheds novel insights on mechanism of differential localization of Dar1 protein, and its effect of dendrite development. The broader implications of these studies are understanding the molecular mechanism of dar-1 transcriptional regulation at a class-specific level and how this regulation ultimately contributes to acquisition of distinct neuronal morphologies that underlie the establishment of complex neural networks.

Abstract 3

CUT MEDIATED TRANSCRIPTIONAL REGULATION OF THE COPII SECRETORY PATHWAY DIRECTS CLASS SPECIFIC DENDRITE MORPHOGENESIS IN DROSOPHILA.

Srividya C. Iyer, Eswar P.R. Iyer, Rama Meduri , Madhu Karamsetty, and Daniel N. Cox, Ph.D.

Department of Molecular and Microbiology, Krasnow Institute for Advanced Study George Mason University, Fairfax, VA 22030 USA

The Drosophila peripheral nervous system (PNS) is a powerful model system in which to investigate the complex processes of neuronal development. Elucidating the molecular mechanisms controlling dendrite development is key to our understanding how neuronal morphologies arise and how they function in achieving synaptic integration and neuronal function. Recent evidence has shown that mutations in select secretory pathway genes preferentially affect dendritic growth. Phenotypic analyses of loss-of-function sec31 mutants, reveal a reduction in dendritic branching indicating a cell autonomous role in mediating da neuron dendritic complexity. Furthermore, gain-of-function analyses indicate sec31 can lead to decrease in dendritic complexity in Class IV da neurons and an increase in complexity in Class I da neurons. Microarray analyses, quantitative RT-PCR and immunohistochemistry experiments reveal that over expression of the homeodomain transcription factor Cut in class I da neurons leads to upregulated expression levels of the components of the COPII-mediated secretory pathway. Microarray analyses and RT-PCR experiments further demonstrate that ectopic over expression of Cut in Class I da neurons also leads to an upregulation in the expression of the transcription factor CrebA, previously implicated in secretory activity of the Drosophila salivary gland. Moreover, simultaneous expression of UAS-Cut and CrebA specific UAS-RNAi elements in Class I da neurons, suppressed the Cut GOF phenotype indicating that CrebA is likely a downstream effector of Cut mediated transcriptional regulation in da neurons. Consistent with this regulatory relationship, overexpression of CrebA in da neurons likewise leads to higher expression levels of components of ER-to-Golgi transport. Collectively, these findings provide novel insight into the role of transcriptional regulation of the COPII-mediated secretory pathway in mediating class specific dendrite morphogenesis.

Abstract 4

CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II BETA (CAMKII-BETA): A REGULATOR OF CNS MYELINATION

Waggener, C.T.[1], Dupree, JL[1], Elgersma, Y.[2], Fuss, B.[1]

1Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, USA. [2]Department of Neuroscience, Erasmus University Medical Centre, Rotterdam, the Netherlands

CNS myelination represents a developmental process that enables fast and efficient signal propagation and maintains axonal integrity. During this developmental process the myelinating cells of the CNS, namely oligodendrocytes, differentiate from bipolar progenitor cells, first into premyelinating oligodendrocytes extending a complex process network and then into fully mature oligodendrocytes generating myelin membranes. Subsequent concentrical wrapping of myelin membranes around axonal segments followed by compaction leads to the formation of a fully functional myelin sheath. During in particular the later stages of myelination, i.e during oligodendrocyte process outgrowth and myelin sheath formation, dynamic changes in the cytoskeleton are thought to play important regulatory roles. However, the exact mechanisms controlling such cytoskeletal dynamics are not fully understood. Our data presented here, suggest that Ca^{2+}/calmodulin-dependent protein kinase II beta (CaMKII-beta) is involved in regulating oligodendrocyte process outgrowth *in vitro* and the formation of a fully developed myelin sheath *in vivo*. More specifically, we have characterized the expression of the four mammalian CaMKII genes and their splice variants in maturing oligodendrocytes. Blocking the function of CaMKII in maturing oligodendrocytes in culture interferes with process outgrowth. Upon siRNA-mediated knock-down of individual CaMKII genes, the effect on process outgrowth is seen only upon knock-down of CaMKII-beta. Interestingly, CaMKII-beta possesses a unique feature not characterized for other CaMKII genes, namely an actin binding site known to limit depolymerization of actin filaments, thus suggesting that CaMKII-beta may be involved in regulating the oligodendrocyte's actin cytoskeleton. To assess CaMKII-beta's role in myelination *in vivo,* we analyzed the ventral spinal cord of developing *Camk2b*$^{-/-}$ mice. These data suggest that *in vivo* CaMKII-beta plays a role in regulating the formation of a fully functional myelin sheath possibly by controlling the oligodendrocyte’s actin cytoskeleton.

Abstract 5

THE ROLE OF NOTCH SIGNALING IN NEUROTRANSMITTER PHENOTYPE SPECIFICATION AND SECONDARY NEUROGENESIS IN *X. LAEVIS*

Molly J. McDonough and Margaret S. Saha

College of William and Mary, Williamsburg, VA

The acquisition of a neurotransmitter phenotype by individual neurons in the developing brain and spinal cord is crucial for the formation of a functioning nervous system in the adult organism. During development, the proliferation of neural progenitor cells and the ultimate differentiation of neurons are tightly regulated to ensure the formation of a properly functioning nervous system. An important signaling pathway involved in this regulation is the Notch pathway, a juxtacrine signaling mechanism that controls the balance between the differentiated and progenitor cells during primary neurogenesis via lateral inhibition. This project aims to determine the role of Notch signaling during the sequential developmental progression of neural differentiation and transmitter specification by exploring two major questions. First, we will examine the effects of early perturbations of Notch signaling on neurotransmitter phenotype specification in embryonic stages of development. Preliminary data from experiments addressing the role of Notch signaling in neurotransmitter phenotype specification indicate that perturbations have significant effects on gene expression in neural fold, neural tube, and tail bud stages of development. However, gene expression at hatching and swimming tadpole stages of development appears to compensate for these earlier effects. The second portion of this project aims to determine the extent to which this compensatory gene expression is occurring using real time PCR. These experiments will aid in elucidating the potential role of Notch signaling in the process of secondary neurogenesis.

Abstract 6

THE FUNCTIONAL CHARACTERIZATION OF $GABA_A$ A AND $GABA_B$ RECEPTOR SUBUNITS IN THE DEVELOPING NERVOUS SYSTEM OF *XENOPUS*

Brian A. Rabe, Gwendolyn E. Kaeser, and Margaret S. Saha

Department of Biology, The College of William and Mary, Williamsburg, VA

The predominating inhibitory neurotransmitter in the adult nervous system, gamma-aminobutyric acid (GABA), acts through two types of receptors. $GABA_A$ receptors are ligand-gated chloride channels while $GABA_B$ receptors are G-protein coupled receptors. In the developing nervous system, GABA acts primarily in an excitatory manner, causing the depolarization of cell membranes. During development, GABA has been implicated as a factor involved in multiple processes including cell migration, proliferation, synapse formation, and neurotransmitter phenotype specification. We therefore predicted that GABA receptor subunits are expressed in embryos at the neurula stages of development which serves as a critical period for neurotransmitter specification. Using *in situ* hybridization and semi-quantitative real time RT-PCR to show spatial and temporal expression of five $GABA_A$ α subunits and the two $GABA_B$ subunits, we have demonstrated that this is indeed the case. In *Xenopus laevis* embryos, each of these subunits is expressed throughout the developing nervous system, in regions including the brain and the spinal cord as well as the retina, pituitary gland, pineal gland, and various cranial nerves during neurula stages. Additionally, the $GABA_A$ α2 subunit was present as maternally derived mRNA in the blastula. Each subunit exhibited a unique expression pattern, implying that these subunits have specific functions and that the expression of each one is specifically regulated both spatially and temporally. In order to determine the function of the $GABA_B$ receptors during development, we have injected antisense morpholino oligonucleotides (MOs) to knockdown translation of the $GABA_B$ subunits in *Xenopus laevis* and *Xenopus tropicalis*. The phenotype resulting from these injections includes a reduction of the anterior nervous system including the brain and retina. *In situ* hybridization analysis shows a decrease in a variety of neural and neurotransmitter phenotype marker genes including neural beta tubulin (NBT), vesicular glutamate transporter (vGLUT), and glutamic acid decarboxylase (GAD).

Abstract 7

THE ROLE OF VOLTAGE-GATED CALCIUM CHANNEL GENES IN NEURO-TRANSMITTER PHENOTYPE SPECIFICATION

Wendy Herbst, Brian Rabe, Zoe Welch, and Margaret Saha

Department of Biology, College of William and Mary, Williamsburg, VA

In addition to their role in neurotransmission in the mature nervous system, voltage-gated calcium channels are implicated in a wide variety of developmental processes. Recent literature has demonstrated the role of activity in specifying neurotransmitter phenotype. Given that the voltage-gated calcium channels $Ca_v1.2$, $Ca_v2.1$, $Ca_v2.2$, and $Ca_v3.2$ are expressed at an appropriate time and place during development, we hypothesize that these channels mediate neurotransmitter phenotype specification. To address this we are employing a morpholino "knockdown" approach to prevent the expression of the calcium channels. Thus far we have employed a morpholino to knockdown Cav2.1. Two-cell *Xenopus laevis* embryos are injected with a morpholino that is designed to bind to the 5' UTR of the mRNA and block translation of this calcium channel. The embryos are then raised to the tadpole stage and observed for any morphological or behavior differences due to the lack of $Ca_v2.1$ channels. The embryos have displayed less swimming movement and an underdeveloped nervous system. We are conducting *in situ* hybridization with these injected embryos to analyze the expression of neurons (both excitatory and inhibitory) and to understand what other genes are affected by knocking down $Ca_v2.1$. We are also examining the upstream regulation of $Ca_v2.1$ by identifying conserved sequences using a bioinformatics approach. We plan to clone these upstream regions and use them to drive the expression of GFP in transgenic organisms.

Abstract 8

OSTEOPONTIN UPREGULATION DURING THE ACUTE PHASES OF REACTIVE SYNAPTOGENESIS IS TRANSCRIPTIONALLY REGULATED

Julie Chan and L.L. Phillips

Department of Anatomy and Neurobiology, Virginia Commonwealth University, Dept. of Anatomy and Neurobiology

Osteopontin (OPN) is a pleiotropic inflammatory cytokine capable of modulating CNS growth and plasticity. As a matrix metalloproteinase substrate, it recruits microglia for debris clearance, making it a potential contributor to reactive synaptogenesis following traumatic brain injury (TBI). Prior microarray analysis showed significant elevation of hippocampal OPN mRNA during adaptive synapse formation induced by unilateral entorhinal cortex (UEC) lesion. Subsequent Western blot and immunohistochemical studies revealed a robust OPN increase 1 and 2d after UEC, and a correlated shift of OPN distribution in the deafferented dentate molecular layer (ML). Confocal imaging confirmed OPN localization in activated microglia of the injured ML, suggesting this cytokine may mediate acute immune pathways to condition the extracellular matrix for synaptogenesis. Notably, in models of maladaptive synaptic plasticity we observed significant attenuation of the acute OPN injury response. Given that OPN may be critical in facilitating adaptive synaptic reorganization following TBI, we extended our studies to determine if acute post-injury increases in OPN expression are transcriptionally regulated. Adult male rats were sacrificed at 1 or 2d post-UEC, and hippocampal or ML RNA extracts evaluated for OPN by qRT-PCR. Significant multi-fold increases in OPN transcript at 1 and 2d post-injury were consistent with transcript elevation seen in preliminary microarray studies. Importantly, these time-dependent increases in hippocampal OPN mRNA matched the post-injury time course of OPN protein elevation in our prior studies. Group qRT-PCR analysis at 1 and 2d also trended toward higher OPN mRNA levels in the deafferented ML compared to whole hippocampus, an effect also observed in the microarray profile. Finally, this robust ML OPN transcript increase correlated with elevated microglial OPN protein at 1 and 2d post-injury, suggesting glia may synthesize OPN during injury-induced synaptogenesis. To confirm microglia as an OPN source, *in situ* hybridization studies are underway to localize OPN transcript within the deafferented hippocampus. Taken together, these results suggest TBI induces rapid OPN transcript elevation which is spatially and temporally synchronous with robust OPN protein production. Further, this response occurs during the acute inflammatory period following injury and is likely mediated through reactive microglia. Future studies comparing OPN expression after UEC with that induced by maladaptive plasticity will provide insight into how this cytokine contributes to successful synaptic recovery following TBI.

Supported by NIH NINDS grants NS44372, NS57758.

Abstract 9

GLIAL AND EXTRACELLULAR MATRIX RESPONSE IN THE INTERNAL CAPSULE FOLLOWING TRAUMATIC BRAIN INJURY

A.E. Doperalski, A.K. Ottens, L.L. Phillips

Department of Anatomy and Neurobiology, Virginia Commonwealth University, Dept. of Anatomy and Neurobiology

Axonal damage is a major feature of traumatic brain injury (TBI). Injured axons interact with reactive glia and the extracellular matrix (ECM) to achieve recovery. The principle cellular mediators of recovery are astrocytes, oligodendrocytes and microglia, secreting repair promoting molecules and inhibitory proteins, such as chondroitin sulfated proteoglycans (CSPGs) and matrix metalloproteinase (MMPs), into the ECM. Prior TBI studies of corpus callosum document time dependent alterations in reactive glia and their expression of ECM molecules critical to recovery. Differential myelinated and unmyelinated fiber recovery was correlated with distinct glial and matrix change. Because TBI affects multiple fiber tracts throughout the brain, it is important to understand glial and ECM response in pathways with different axon composition. The internal capsule is a tract composed principally of large caliber, myelinated fibers with multiple targets in the brain, brainstem, and spinal cord. Using the central fluid percussion model of diffuse TBI, we examined glial cell response in the internal capsule at 1 and 3 days post-injury using confocal immunohistochemistry with antibodies specific for astrocytes, oligodendrocytes, or microglia. To explore ECM mediation of axonal recovery, we assessed CSPG co-localization within each glial type. Altered microglial and astrocyte morphology was observed at 3 d. Microglia were reactive, with elongated somas and short, thick processes. Hypertrophic astrocytic processes had increased filament staining. In contrast, oligodendrocyte response appeared at 1 d, with cell body redistribution along axon bundles. CSPGs co-localized within reactive astrocytes. Western blot analysis of internal capsule αII spectrin lysis revealed increase of calpain and caspase-3 derived fragments at 3d. Together, these data support complex glial reactivity in the internal capsule within 3 d after TBI, a response which correlates with significant αII spectrin breakdown. Further, CSPG localization in reactive astrocytes links ECM cues with the process of axonal recovery. Supported by: NIH-NS4437, NS57758

Abstract 10

NF-κB P50 REGULATES SYSTEMIC INFLAMMATION AND NEUROINFLAMMATION IN AGING

Thomas Taetzsch, Michael Surace, Shannon Levesque, Constance McGraw, Laura Duke & Michelle Block

Department of Anatomy and Neurobiology, Virginia Commonwealth University Medical Campus, Richmond, VA 23298, USA

Inflammation associated with chronically activated microglia has been implicated in the progressive degeneration of nigral dopaminergic (DA) neurons in Parkinson's disease. While aging has been linked to neurotoxic microglial activation, the mechanisms are poorly understood. To examine the role of NF-κB p50 in microglial activation in vivo, NF-κB p50+/+ and NF-κB p50-/- mice were injected with 5mg/kg LPS IP and sacrificed after 3 hours. TNFα and IL- 1β mRNA expression and activated microglia morphology in the brains of LPS treated NF-κB p50-/- mice were significantly more than that of NF-κB p50+/+ mice. In vitro studies revealed that both activated microglia morphology and TNFα levels in response to LPS (100ng/ml) were also higher in NF-κB p50-/- mesencephalic neuron-glia cultures, indicating that NF-κB p50-/- glia are more sensitive to pro-inflammatory stimuli. To address the role of NF-κB p50 in microglia- mediated DA neurotoxicity, primary NF-κB p50+/+ and NF-κB p50-/- cultures were treated with lipopolysaccharide (LPS 0,10, or 100 ng/ml). NF-κB p50-/- cultures showed enhanced DA neurotoxicity when compared to NF-κB p50+/+ cultures. To discern the role of NF-κB p50 in aging and inflammation, NF-κB p50+/+ and NF-κB p50-/- mice from four age groups (1.5-3, 4-6, 8- 11.0 or 16-18.0 months) were injected with 5mg/kg LPS or saline IP and sacrificed after 3 hours. Serum TNFα levels in LPS treated NF-κB p50-/- mice were higher than NF-κB p50+/+ mice in all age groups and this difference precipitously increased in older mice (8-11 and 16-18 months), suggesting that NF-κB p50 has an increasingly important role in inflammation with age. These studies underscore the role of NF-κB p50 in systemic inflammation, neuroinflammation and microglial activation in aging.

Abstract 11

AIR POLLUTION-INDUCED NEUROPATHOLOGY: PETROLEUM DIESEL AND BIODIESEL EXHAUST CAUSE NEUROINFLAMMATION AND MICROGLIAL ACTIVATION

Surace M, Levesque S. Ramirez J, Kodavanti U, Kodavanti P, Roland J*, & Block ML

Department of Anatomy and Neurobiology, Virginia Commonwealth University Medical Campus, Richmond, VA 23298, USA

*National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC 27711, USA.

Air pollution has been increasingly linked to CNS disease, but the mechanisms driving this response and the type of exposures responsible are unknown. Microglia, the resident innate immune cell in the brain, have been implicated in the progressive nature of neurodegenerative disease, but their role in air pollution-induced neuropathology is poorly understood. Using diesel engine exhaust inhalation as a model of urban air pollution, here we explore the ability of both petroleum diesel (DE) and biodiesel (BDE) to induce neuroinflammation, microglial activation, and DA neurotoxicity in the substantia nigra (SN). Wistar Kyoto (WKY) and Spontaneously Hypertensive (SHR) rats were exposed by inhalation for 1 month at 0, 50, 150, or 500 μg/m3 BDE. WKY rats were exposed by inhalation for 1 month at 0, 500, or 2000 μg/m3 DE and Sprague Dawley rats were instilled intratracheally (IT) with diesel exhaust particulate (DEP) (SRM 2975; 20 mg/kg). Rats exposed to DE by inhalation show increased neuroinflammation (nitrotyrosine, IL-6, IBa1, TNFα, MIP-1α) particularly in the midbrain (location of SN). IT DEP induced serum as well as brain TNFα as well as morphological activation of microglia. In response to BDE inhalation, microglia display activated morphology, yet classical markers of activation such as TNFα and nitrotyrosine protein and IL-6 mRNA are conspicuously absent. Together these data indicate that microglia do indeed detect and respond to DE and BDE, but the activation profile for each exposure is unique.

Abstract 12

POTENTIAL ROLE OF THE μ-OPIOID-/NOCICEPTIN ORPHANIN FQ RECEPTOR SYSTEM IN DEVELOPMENTAL MYELINATION AND MULTIPLE SCLEROSIS

Vestal-Laborde, AA[1], Eschenroeder, AC[1], Dupree, JL[2], Bigbee, JW[2], De Vries, GH[3], Robinson, SE[4], Sato-Bigbee, C.[1]

[1] Biochemistry and Molecular Biology, [2] Anatomy and Neurobiology, [4] Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University and [3] McGuire VA Medical Center, Richmond, VA, USA

While the classical function of myelin is to facilitate saltatory conduction, this membrane and the myelin-making oligodendrocytes (OLGs) are now recognized as regulators of plasticity and remodeling in the central nervous system (CNS). As such, OLG maturation and myelination are among the most vulnerable processes along CNS development and are also affected in demyelinating diseases such as multiple sclerosis (MS). We previously showed that rat brain myelination is altered by perinatal exposure to buprenorphine (BUP), an opioid analogue currently in clinical trials for the treatment of pregnant opioid addicts. Therapeutic BUP doses induced accelerated and increased expression of all splicing variants of myelin basic proteins (MBPs), cellular and myelin components that are markers of mature OLGs. In contrast, supra-therapeutic drug doses delayed MBP brain expression and resulted in a decreased number of myelinated axons. Using cultured OLGs, we recently found that the *in vivo* effects on myelination can result from direct alteration in the balance between μ-Opioid Receptor (MOR) and Nociceptin Orphanin FQ Receptor (NOPR) activities. MOR stimulation results in OLG differentiation, while this positive effect is counteracted by an inhibitory response mediated by NOPR. These observations suggest that a delicate balance between MOR and NOPR signaling plays a crucial role in the timing of OLG maturation and myelin formation. This finding is particularly intriguing because the NOPR/nociceptin system has been linked to behavior and pain regulation but a role in CNS development has not been described. Interestingly, we found that NOPR ligand nociceptin is increased in rat and human astrocytes in response to treatment with inflammatory cytokines which are elevated in MS. Furthermore, our preliminary results also showed elevated nociceptin levels in experimental allergic encephalomyelitis (a model of MS) and in brain samples of MS patients. Altogether, our findings suggest that exposure to opioids may disrupt the normal interplay between MOR and NOPR, altering the developmental pattern of brain myelination. Furthermore, disruption of this equilibrium at sites of demyelination in MS could interfere with the replenishment of mature cells capable of remyelination.

Abstract 13

FUNCTIONAL INTERDEPENDENCE OF P75-NGFR AND TNFR1 DURING SYMPATHETIC NERVOUS SYSTEM DEVELOPMENT

Kazusa Edamura, Kelvin Chan, Danielle Heffner, Catherine Jansch, Christopher Dawson and Christopher Deppmann

Department of Biology, University of Virginia, Department of Biology

The sculpting of the peripheral nervous system relies on functionally antagonistic neurotrophin pathways. Target-initiated Trk signaling mediates cell survival, axon growth, and synapse formation. In contrast, p75-NGFR mediates opposing functions such as cell death, axon degeneration, and synapse restriction. p75 is part of the tumor necrosis factor receptor (TNFR) superfamily, of which some members have been reported to have roles similar to p75 in peripheral nervous system development. In the sympathetic nervous system, 3 of these receptors, p75, TNFR1, and DR6 are highly expressed. Here we demonstrate that p75 and TNFR1 promote cell death and axon growth inhibition in vitro, which our in vivo analysis of cell number and target innervation also supports and suggests there's a synergy effect between these receptors. What is the logic of having paralogous receptors expressed at the same place and time performing identical tasks? Are they functionally redundant or perhaps interdependent? To address this question, we cultured sympathetic neurons from p75 or TNFR1 null animals and exposed them to BDNF (to activate p75) or TNFα (to activate TNFR1) and assayed axon growth and cell death. We found that the answer to the redundancy versus interdependency question is dependent on functional context. In the context of axon growth inhibition, p75 and TNFR1 appear to be somewhat functionally redundant, since BDNF can inhibit axon growth in the absence of TNFR1. However, in the context of cell death these receptors require one another to function since BDNF or TNFα cannot promote cell death in the absence of TNFR1 or p75, respectively. This novel aspect of p75/TNFR signaling may provide an avenue to examine the basis for cross-talk with NGF-TrkA. When p75-/-, TNFR1-/- and DKO (p75-/-;TNFR1-/-) neurons are exposed to several NGF levels, each genotype exhibits distinct cell survival curves. We also demonstrate that NGF-TrkA can alter the subcellular localization of p75 in mature neurons, which suggests a basis of cross-talk in the aforementioned functional contexts as well as synapse formation (Sharma et al.).

Abstract 14

THE ROLE OF SULFATIDE IN THE DEVELOPMENT AND MAINTENANCE OF NODAL AND PARANODAL DOMAINS IN THE PERIPHERAL NERVOUS SYSTEM

Heather F. Herman, Jeffrey L. Dupree

Department of Anatomy and Neurobiology, Virginia Commonwealth University Medical Center, Richmond, VA

Sulfatide is a galactolipid and a major lipid component of the myelin sheath. Its production is catalyzed by the enzyme cerebroside sulfotransferase (CST). To determine the functions of sulfatide, the gene encoding CST was genetically disrupted resulting in mice incapable of sulfatide synthesis. Using these mice, it has been shown in the central nervous system (CNS) that sulfatide is required for proper initial clustering of paranodal proteins and cluster maintenance of nodal proteins suggesting that paranodal domains are important for long-term node stability. In contrast to the CNS, a requirement for sulfatide in the initial paranodal and nodal clustering or in the long-term maintenance of these clusters in the peripheral nervous system (PNS) has not been analyzed. Therefore, we have employed a combination of immunocytochemistry and confocal microscopic analysis of the CST null mice to determine the role of sulfatide in cluster onset and maintenance in the PNS. For these studies we have quantified the number of clusters of both paranodal and nodal proteins in the CST null and wild type mice at 4 days, 7 days, 15 days, 1 month, and 10 months of age. Preliminary findings suggest that the paranodal myelin protein neurofascin 155 (Nfasc155) shows a decrease in initial clustering in the CST null mice at 4 days of age and the neuronal voltage-gated sodium channels show a decrease in clustering at 7 days of age. Clustering of the paranodal neuronal protein contactin does not show this same decrease at 4 days of age; however, contactin is shown to be significantly decreased in the CST null mice at 15 days and 1 month of age. Gliomedin clustering in the CST null mice is seen to be decreased at 1 month of age. Nfasc155 clustering in the CST null mice remains decreased compared to the WT mice at 4 days, 15 days, and 1 month of age, but this deficit is resolved by 10 months of age. In addition, our ultrastructural analysis confirms that paranode formation is compromised in young mice as transverse bands are rarely observed in the absence of sulfatide. Together, our findings demonstrate that sulfatide is essential for the appropriate temporal onset of nodal and paranodal protein clustering and paranodal structural formation in the PNS. Although clustering onset is delayed, normal clustering of both nodal and paranodal proteins is achieved demonstrating that sulfatide is not an essential component of nodal/paranodal clustering nor is it required for long-term maintenance. Finally, in contrast to previous reports, our findings indicate that nodal stability is independent of paranodal integrity.

Abstract 15

OPIOID ANALGESIC INFLUENCE ON THE EXPRESSION OF CONDITIONED FEAR IN C57BL/6J MICE, POTENTIALLY PREDICTIVE OF THEIR ROLE IN PTSD.

S A Vunck[1], S E Snider[2], PM Beardsley[2]

[1]Department of Psychology, Virginia Commonwealth University, Richmond VA, [2]Department of Pharmacology and Toxicology Virginia Commonwealth University, Richmond VA

Aims: Posttraumatic Stress Disorder (PTSD) is an anxiety disorder that affects over 7.7 million adults and carries an estimated societal cost of $6 billion. Comorbidity for opiate abuse and PTSD has been reported. It is important to understand the impact of opioid administration on the disorder acutely and long term.

Methods: This study presents data from mice that have undergone Pavlovian fear conditioning and a single contextual exposure session. The assay was conducted over three days; a conditioning day, an exposure day, an extinction learning test day. Test compounds were administered 20 minutes prior to day 2 exposure session.

Results: Morphine significantly reduced contextual freezing acutely in 3 mg/kg ($p < 0.01$) and 10 mg/kg ($p < 0.001$) groups. Morphine significantly facilitated extinction of contextual freezing in the 1 mg/kg, 3 mg/kg and 10 mg/kg groups ($p < 0.01$). Fentanyl significantly reduced contextual freezing acutely in the 0.01 mg/kg group ($p < 0.001$) and facilitated extinction of contextual freezing at all doses 0.001 mg/kg, 0.01 mg/kg and 0.1 mg/kg ($p < 0.001$). Buprenorphine significantly reduced contextual freezing acutely at all doses 0.3 mg/kg, 1 mg/kg and 3 mg/kg ($p < 0.001$) but did not significantly affect extinction. Naloxone did not significantly affect acute contextual freezing behavior or extinction.

Conclusions: These results support that the use of analgesics like morphine and fentanyl in the treatment of trauma could have an added benefit of reducing the eventual severity of PTSD. It may also help explain the comorbidity of opioid abuse in PTSD patient populations. Buprenorphine which is used in the treatment of substance abuse may result in acute relief of some PTSD symptoms but will probably not have a long term effect. Naloxone will most likely have no effect on symptoms of the disorder acutely or long term.

Financial Support: NIDA training grant T32DA007027

Abstract 16

NEW LEAD COMPOUND IN SERIES OF BIVALENT MULTIFUNCTIONAL LIGANDS AS Aß OLIGOMERIZATION INHIBITORS

Jeremy Chojnacki, Dr. Kai Liu, and Dr. Shijun Zhang

Department of Medicinal Chemistry, School of Pharmacy, VCU

Purpose: Alzheimer's disease is a widespread neurodegenerative disorder affecting more than 24 million people worldwide. There is a large body of evidence suggesting that the aggregation of amyloid-ß (Aß) oligomers into plaques and an increase in oxidative stress contribute significantly to development of the disease. Furthermore, recent studies have indicated neuronal cell membrane lipid rafts as a key site for Aß production and oligomerization. Based on these findings and our previous series of ligands, our purpose is to synthesize new compounds targeting Aß, oxidative stress, and lipid rafts as potential therapeutic and/or diagnostic agents for Alzheimer's disease. **Methods:** We followed a logical synthetic route in making the first in a new series of compounds, utilizing the increasingly popular azide-alkyne click reaction. Furthermore, preliminary cell viability was assayed using MC65 neuroblastoma cells. **Results:** Based on our design, we successfully synthesized the first in a new series of compounds, seen in Figure 1. With this new compound, we can further validate our proof of concept and examine the effect of linker composition on *in vitro* activity. Our ligand consists of three distinct parts in order to satisfy our design criteria: a cucurmin moiety, a cholesterol moiety, and a linker joining the two.

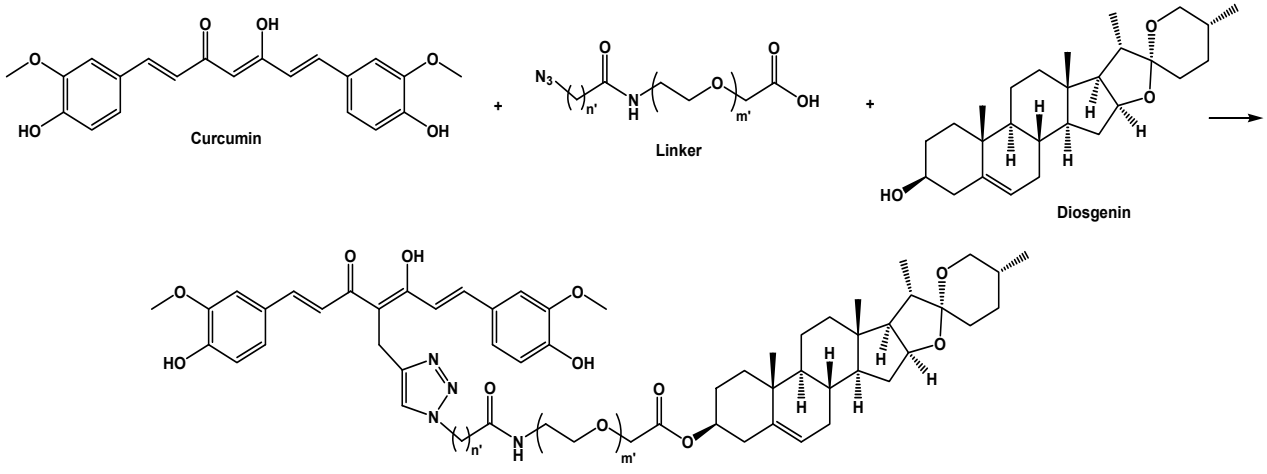

Figure 1 – Synthesis of First Compound of New Series, m'=3, n'=5

Conclusion: With positive cell viability results, we are currently conducting more *in vitro* assays and are hopeful this new compound will display strong anti-oligomerization properties. In addition, we are also synthesizing more members of this series to examine the *in vitro* effects of linker composition and length.

IBUDILAST, MINOCYCLINE, AND AV1013 ATTENUATE METHAMPHETAMINE INTRAVENOUS SELF-ADMINISTRATION IN RATS

Sarah E. Snider1, S. A. Vunck1, E. Hendrick1, E. J.C.G. van den Oord2, D. E. Adkins2, J. L. McClay2, P. M. Beardsley1,2

1Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 2Department of Pharmacy, Virginia Commonwealth University, Richmond, VA

Methamphetamine (METH) is an abused psychomotor stimulant that causes hyperactivity and feelings of euphoria which can lead to continuous drug-seeking and chronic use. While METH abuse leads to a plethora of untoward effects, and costs society billions of dollars each year; METH abuse is still without an approved medication for the approximately 800,000 current users. Intravenous (i.v.) self-administration is an established model of drug-taking behavior and METH self-administration is well maintained in laboratory animals. Ibudilast (AV411; 3- isobutyryl-2-isopropylpyrazolo-[1,5-a]pyridine) inhibits phosphodiesterase (PDE) and glial pro- inflammatory activity, both effects thought to potentially blunt METH-induced behaviors. We have previously reported ibudilast to block stress- and primed- induced reinstatement of METH drug-seeking in rats as well as METH-induced locomotor activity and sensitization in mice. Two additional compounds, Minocycline, a tetracycline derivative, and AV1013, an amino-analog of ibudilast, have minimal PDE inhibitory activity but suppress METH-induced glial activation. In an effort to more directly examine the effect of glial modulation on METH's abuse-like behavioral effects, the present study determined whether ibudilast, minocycline, or AV1013 could attenuate METH i.v. self-administration in Long-Evans hooded rats. Ten rats were implanted with indwelling intravenous catheters, and initially trained to respond on a lever for 0.1 mg/kg/inf METH (0.2 ml in 6 s infusion + 14 s TO) according to FR1 reinforcement schedules during 2-h daily sessions. Once stable responding was reached, twice daily ibudilast (1, 7.5, 10 mg/kg), once daily minocycline (10, 30, 60 mg/kg), or twice daily AV1013 (1, 10, 30 mg/kg) i.p. administrations were given on three consecutive days of METH (0.001, 0.03, 0.1 mg/kg/inf) self- administration. All three compounds significantly ($p<0.05$) reduced responding for the intermediate dose of METH self-administration (0.03 mg/kg/inf). As neither minocycline nor AV1013 have potent PDE-related activity, these results suggest that targeting glial cells may be sufficient for attenuating METH drug-taking behavior which may provide a potential mechanism for pharmacotherapies treating METH abuse and its relapse. This research was supported by Virginia Commonwealth University's Institute for Drug and Alcohol Studies and Center for Biomarker Research and Personalized Medicine.

Abstract 18

A POPULATION ACTIVITY MODEL OF CORTICO-STRIATAL CIRCUITRY UNDERLYING BEHAVIORAL INHIBITION IN RATS.

Hardcastle, K.; Smith, G.D.; Burk, J.A.

Departments of Applied Science and Psychology, The College of William & Mary, Williamsburg, VA 23187

Impulsivity, often characterized as a failure of inhibition, critically contributes to the symptoms of several psychological disorders, including attention deficit hyperactivity disorder and drug use. Failure of inhibition can be assessed with tasks that require the ability to stop an initiated response, such as the stop signal reaction time task. Numerous studies have implicated the basal ganglia in action selection and motor control. We constructed a neural network modeling framework that can assess the specific neurobiological mechanisms subserving impulsivity, consistent with known anatomy and physiology of the basal ganglia. Our minimal formulation includes six groups of nuclei: the cortex, striatal D1 receptor expressing neurons, striatal D2 receptor expressing neurons, the globus pallidus, the subthalamic nucleus (STN), and the substantia nigra reticulum (SNr). A stochastic Wilson-Cowan-type system of nonlinear differential equations depicts the spiking and synaptic activity of these neural populations. Three different channels represent subpopulations of neurons corresponding to actions that may be selected, as determined by suppression of SNr activity. Parameter studies are performed by analyzing model predictions of action selection probability, and these results are compared with similar models of action selection within the basal ganglia. In addition, our model accurately replicated general dynamics seen in the SSRT: the probability of accurately inhibiting a response decreases approximately linearly as the stop signal delay is increased.

Abstract 19

EFFECTS OF ACUTE NICOTINE ON DELAY DISCOUNTING IN RATS.

Haque S, Agate FT, Bataller H, Wolfe E, Burk JA

Dept. of Psychology, The College of William & Mary, Williamsburg VA 23187.

Delay discounting, reflects the degree to which a reward is devalued as the delay to receive reward access is increased. The effects of nicotine on delay discounting hav been mixed, with some studies suggesting nicotine increases preference for a large, delayed reward while other studies report that nicotine increases preference for a smaller, immediate reward. These differences may depend on numerous task variables. One variable that has not been examined is the impact of using shorter delays on any nicotine-induced effects. In the present experiment, Male FBNF1 hybrid rats were trained in a delay discounting task involving access to a smaller reward (0.01 ml of tap water) and a larger reward (0.06 ml of tap water). The delay to large reward access was progressively increased during a session (0, 3, 6, 9, 12 s) After achieving stable performance levels, rats received nicotine (0.0, 0.10, 0.20, 0.40 mg/kg, ip) in a randomized order 10 min prior to task performance. Compared to saline administration, 0.20 mg/kg nicotine increased the probability of selecting the immediately available, low reward. The highest nicotine dose increased the number of trials in which the animals failed to select either reward. The present data are consistent with findings that nicotine can increase preference for small, immediate rewards, suggesting increased impulsivity. The present experiment extends these findings to delay discounting procedures with relatively short delays to large reward access. Supported by AG030646.

Abstract 20

EFFECTS OF INTRABASALIS OREXIN A ON ATTENTIONAL PERFORMANCE.

Zajo KN, Yanev P, Fujita A, Burk, JA

Dept. of Psychology, The College of William & Mary, Williamsburg VA 23187.

Orexins are neuropeptides released from neurons that are primarily located in the hypothalamus and contiguous perifornical area. Orexinergic neurons project to several brain regions including to the basal forebrain. The basal forebrain is necessary for normal attentional processing in rats. Our previous research demonstrated that orexin receptor blockade impairs attention. The goal of the present experiment is to test whether the basal forebrain mediates the attention-enhancing effects of orexin A. Male FBNF1 hybrid rats were trained in a visual signal detection task that requires discrimination of brief visual signals (500, 100 or 25-ms) from trials when no signal is presented. After stable performance levels were established, rats bilateral guide cannula were implanted into the basal forebrain. Postsurgically, rats were trained in a version of the task in which attentional demands were augmented by presenting a visual distracter during the middle block of trials. Although preliminary, our findings provide evidence that intrabasalis orexin A administration may decrease distracter-induced deficits in attention task performance, specifically on nonsignal trials. Our findings to date support the conclusion that orexin A may benefit attentional processing, specifically through projections to the basal forebrain. Supported by AG030646.

Abstract 21

ROLE OF ADRENAL GLUCOCORTICOID SIGNALING IN PREFRONTAL CORTEX GENE EXPRESSION AND ACUTE BEHAVIORAL RESPONSES TO ETHANOL

Blair N. Costin, Aaron R. Wolen, Sylvia Fitting, Keith L. Shelton, and Michael F. Miles

Department of Pharmacology and Toxicology, Virginia Commonwealth University Richmond, VA, USA

Background: Glucocorticoid hormones modulate acute and chronic behavioral and molecular responses to drugs of abuse including psychostimulants and opioids. There is growing evidence that glucocorticoids might also modulate behavioral responses to ethanol. Acute ethanol activates the HPA axis, causing release of adrenal glucocorticoid hormones. Our prior genomic studies suggest glucocorticoids play a role in regulating gene expression in the prefrontal cortex (PFC) of DBA2/J (D2) mice following acute ethanol administration. However, few studies have analyzed the role of glucocorticoid signaling in behavioral responses to acute ethanol. Such work could be significant, given the predictive value for level of response to acute ethanol in the risk for alcoholism.

Methods: We studied whether the glucocorticoid receptor (GR) antagonist, RU-486, or adrenalectomy (ADX) altered male D2 mouse behavioral responses to acute (locomotor activation, anxiolysis or loss-of-righting reflex (LORR)) or repeated (sensitization) ethanol treatment. Whole genome microarray analysis and bioinformatics approaches were used to identify PFC candidate genes possibly responsible for altered behavioral responses to ethanol following ADX.

Results: ADX and RU-486 both impaired acute ethanol (2 g/kg) induced locomotor activation in D2 mice without affecting basal locomotor activity. However, neither ADX nor RU-486 altered initiation of ethanol sensitization (locomotor activation or jump counts), ethanol-induced anxiolysis or LORR. ADX mice showed microarray gene expression changes in PFC that significantly overlapped with acute ethanol-responsive gene sets derived by our prior microarray studies. Q-rtPCR analysis verified that ADX decreased PFC expression of *Fkbp5* while significantly increasing *Gpr6* expression. In addition, high dose RU-486 pre-treatment blunted ethanol-induced *Fkbp5* expression.

Conclusions: Our studies suggest that ethanol's activation of adrenal glucocorticoid release and subsequent GR activation may partially modulate ethanol's acute locomotor activation in male D2 mice. Furthermore, since adrenal glucocorticoid basal tone regulated PFC gene expression, including a significant set of acute ethanol-responsive genes, this suggests that glucocorticoid regulated PFC gene expression may be an important factor modulating acute behavioral responses to ethanol.

Abstract 22

INVESTIGATING ALTERATIONS TO THE SYNAPTIC TRANSCIPTOME IN RESPONSE TO ETHANOL EXPOSURE.

M.A. O'Brien; M.F. Miles.

Virginia Commonwealth University, Department of Pharmacology/Toxicology, Richmond, Virginia, VA 23233.

It is suggested that the characteristic behaviors associated with the escalation of drug use are caused by molecular adaptations precipitated by the drug's continual administration. These lasting activity-dependent changes depend on new protein synthesis and remodeling at the synapses. It is well established that mRNA can be transported to neuronal distal processes, where it can undergo localized translation regulated in a spatially restricted manner in response to stimulation. These concepts have lead to our hypothesis that behavioral sensitization in response to repeated ethanol exposure results, at least in part, from alterations in the trafficking of mRNAs to distal processes, contributing to synaptic remodeling and plasticity. To identify molecular targets involved in synaptic plasticity, we have optimized a protocol for obtaining synaptoneurosomes from the frontal pole of mice treated with repeated ethanol. In this protocol, fresh tissue undergoes homogenization and fractionation resulting in a pelleted fraction (P2) that should be enriched with vesicularized pre- and post-synaptic elements. Characterization of the preparation through, transmission electron microscopy, western blotting and quantitative PCR indicates synaptic enrichment. Analyzing the distribution of total RNA isolated from synaptoneurosomal fractions also revealed an enrichment of small molecular weight RNAs present in the P2 pellet. Subsequent microRNA qPCR analysis indicated that this enrichment correlated with a significant increase in the level of microRNA species. Preliminary investigations of the synaptic transcriptome utilizing microarray analysis of tissue from mice chronically exposed to ethanol also revealed an overrepresentation of transcripts denotative of synaptic function in fractions enriched in synaptic elements. These experiments also revealed regulation of synaptic gene expression by ethanol. Genes such as Kcnma1, Rbm9, Gabra2, and Gsk3B were all found to be up-regulated in mice chronically consuming ethanol. We therefore conclude that the synaptoneurosome preparation will provide us with samples enriched in synaptically localized mRNAs and proteins that will aid our investigation into the underlying molecular alterations that contribute to behavioral sensitization in response to repeated ethanol.

Abstract 23

A SPECIFIC ROLE FOR THE RHO1 PATHWAY IN THE *DROSOPHILA* NEURAL CLOCK CIRCUIT

Neethi Rao, Ariel Talts, Rachel Siegmund, Laura Thomason, Emmanuel Anyetei-Anum, and Herman Wijnen

Department of Biology, University of Virginia, Charlottesville, VA, USA

Daily rhythms in behavior and physiology are a conserved property of many organisms. Generation of these rhythms is dependent on many cellular and biochemical processes including signaling cascades and regulation of gene expression. We discovered that a conserved signaling pathway associated with modulation of the actin cytoskeleton acts in the *Drosophila* neural clock circuit to control circadian locomotor activity. Transgenic knockdown of the gene encoding the small GTPase *Rho1* (the ortholog of mammalian RhoA) and each of seven genes encoding upstream or downstream effectors of RHO1 signaling resulted in either loss of behavioral rhythms or a lengthened behavioral period length. In-depth analyses for a number of these phenotypes indicated that (1) they were not associated with cell death or gross morphological changes in clock neurons, (2) they could be mapped to the adult ventral lateral clock neurons (LNvs) that express the neuropeptide PIGMENT DISPERSING FACTOR (PDF), and (3) they specifically affected oscillator function in clock neurons rather than peripheral clocks. A molecular characterization in clock neurons of the phenotype associated with knock-down of RHO KINASE (ROK), which acts as a downstream effector of RHO1, revealed a specific delay in nuclear entry of the core clock proteins PERIOD (PER) and TIMELESS (TIM). Taken together, our results show that both molecular and behavioral rhythms generated by the *Drosophila* neural clock circuit exhibit a particular requirement for RHO1 signaling.

Abstract 24

A NEW PROMOTER ELEMENT ASSOCIATED WITH DAILY TIME KEEPING IN *DROSOPHILA*

Brandi Sharp[1], Chris Uyehara[1], Raisa Saab[1], Felix Naef[2], Herman Wijnen[1]

[1]Department of Biology, University of Virginia, Charlottesville, VA, USA
[2]Swiss Institute of Bioinformatics and EPFL, Lausanne, Switzerland

Circadian clocks are autonomous daily time keeping mechanisms that allow organisms to adapt their behavior to environmental rhythms as well as organize biological functions in a coherent daily schedule. The clocks of both humans and fruit flies involve extensive regulation of rhythmic gene expression. To date, relatively few promoter elements have been identified and characterized as associated with clock-controlled gene expression. We recently discovered a 29-bp consensus sequence element conserved in core clock gene promoters across 12 species of *Drosophila*. This element, tentatively termed 'CATAC,' is highly represented in the promoters of clock-controlled genes such as *Par-domain protein 1* (*Pdp1*) and *Slowpoke binding protein* (*Slob*). To experimentally address the spatiotemporal expression information associated with this element, we generated constructs with four separate native CATAC elements from the *Slob* or *Pdp1* promoters upstream of a basal promoter driving expression of either the yeast *Gal4* or firefly *luciferase* reporter genes. Reporter assays showed that presence of wild-type, but not mutant CATAC elements imparted increased expression levels as well as rhythmic regulation. In contrast, the spatial expression patterns of wild-type and mutant CATAC reporters were relatively similar. Part of the CATAC consensus sequence resembles the E-box binding site for the core circadian transcription factor CLOCK/CYCLE (CLK/CYC) and CATAC-mediated expression rhythms are lost in the presence of the cyc^{01} mutation. Nevertheless, CLK/CYC does not appear to act as the predominant direct regulator of the CATAC element. Other transcription factors that may be responsible for mediating CATAC enhancer activity were suggested by binding site prediction as well as a systematic one-hybrid screen. We will discuss our evaluation of these candidates as novel regulators of circadian gene expression.

Abstract 25

A NEW ROLE FOR THE *DROSOPHILA* CIRCADIAN REGULATOR CLOCK/CYCLE IN PUPAL PACEMAKER NEURONS

Karolina Mirowska, Tadahiro Goda, Jake Currie, Min-Ho Kim, Virginia Baker, Gloribel Bonilla, Neethi Rao, Albert Nieh, and Herman Wijnen

Department of Biology, University of Virginia, Charlottesville, VA, USA

Endogenous circadian clocks allow organisms to anticipate daily environmental changes and organize a wide array of biological functions in a daily schedule. The *Drosophila* circadian clock mechanism features a conserved negative feedback loop of gene expression involving the transcription factor CLOCK/CYCLE (CLK/CYC) and its inhibitor PERIOD (PER). Whereas the components and circuits of the adult clock mechanism have been extensively studied, relatively little is known regarding the underlying developmental requirements. Our recent experiments show that adult circadian behavior does not require either a ticking clock or the expression of *per* during prior development. However, inhibition of CLK/CYC activity during metamorphosis either by depletion of CYC or over-expression of PER irreversibly affects clock-controlled locomotor activity in adult flies. In particular, loss of CLK/CYC activity in the developing small ventral lateral clock neurons (s-LN_vs), which express the neuropeptide PIGMENT DISPERSING FACTOR (PDF), leads to neuro-anatomical and molecular phenotypes as well as adult behavioral arrhythmia. In vivo luciferase reporter assays of circadian gene expression indicate that adult peripheral clock function, in contrast, does not depend on developmental CLK/CYC activity. Thus, the newly discovered developmental function for CLK/CYC can be separated from its direct role in the circadian oscillator and it appears to be specific to the neural clock circuits.

PURIFICATION AND RECONSTITUTION INTO LIPID BILAYERS OF THE HUMAN DOPAMINE TRANSPORTER

YooRi Kim, Edgar Leal-Pinto, Louis J. DeFelice

Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, VA

The human dopamine transporter (hDAT) provides the primary mechanism for dopamine clearance in synapses and thus facilitates the regulation of dopaminergic functions in cognition and reward. It is the molecular target of many centrally-active agents including amphetamines, cathinones, and cocaine. Therefore, an understanding of hDAT function and its modulation by these therapeutic drugs and drugs of abuse can provide insight into the mechanisms of abuse and addiction. In the presented studies, hDAT is tagged with a hexahistidine construct and heterologously expressed in *Xenopus laevis* oocytes. The plasma membranes are isolated, solubilized, and applied to a Nickel affinity column to obtain purified hDAT with preserved functionality. Purified hDAT reconstituted in planar lipid bilayers exhibit channel behaviors at physiological membrane potentials. We observe that the current mediated by single hDAT molecules is (1) induced by dopamine and amphetamine, (2) dependent on the sodium electrochemical gradient, and (3) blocked by cocaine in a dose-dependent manner. Our data support hDAT channel activity that is associated with dopamine uptake and presents a novel electrophysiological approach to studying monoamine transporter function and modulation by drugs.

Abstract 27

THE HUMAN SEROTONIN TRANSPORTER EXHIBITS A PERSISTENT LEAK CURRENT

Ernesto Solis, Jr. and Louis J. DeFelice

Department of Physiology and Biophysics, Virginia Commonwealth University

Amphetamine and related compounds increase serotonin (5HT) levels in the brain and cause profound behavioral effects. One target for these drugs is the serotonin transporter (SERT), which normally regulates synaptic 5HT levels. SERT agonists, such as 5HT and amphetamine, induce SERT-mediated currents coupled to Na^+. We employed two-electrode voltage-clamp to measure SERT currents on *Xenopus laevis* oocytes voltage-clamped to -60 mV. We discovered a leak SERT current induced by exposure to amphetamine that persists long after its removal. In this work, we compare the amphetamine-induced leak current in SERT to responses by several amphetamine derivatives including methylenedioxymethamphetamine (MDMA), para-chloroamphetamine (pCA), and methedrone. Understanding this novel effect of amphetamine-related drugs on SERT has implications in the understanding of human behavior.

Abstract 28

BATH SALTS' DOUBLE ACTION AT THE DOPAMINE TRANSPORTER

Krasnodara N. Cameron[1], Renata Kolanos[2], Ernesto Solis[1], Rakesh H. Vekariya[2], Richard A. Glennon[2], Louis J. De Felice[1]

[1] Physiology and Biophysics School of Medicine
[2] Medicinal Chemistry, School of Pharmacy
Virginia Commonwealth University, Richmond, VA, USA.

Cathinone is a naturally occurring psychoactive stimulant found in the leaves of the shrub *Catha edulis*. *Bath salts,* a new drug combination that has invaded the US clandestine market, is a mixture of cathinone analogues, the two main ingredients of which are mephedrone (MEPH) and methylenedioxypyrovalerone (MDPV). Using heterologous expression of human dopamine transporter (hDAT) in *Xenopus* oocytes and two-electrode voltage clamp, we investigated the action of MEPH and MDPV separately and in combination at hDAT. These data show that MEPH has the same electrical signature as AMPH and METH, which are known DAT stimulants. In contrast, MDPV has the signature of COC, a known DAT blocker. These two cathinone analogues should each increase extracellular DA at dopaminergic synapses albeit through different mechanisms: when applied simultaneously (as in *bath salts*), MEPH should release DA while MDPV should prevent its reuptake. We conclude that the physiological and neurological effects of *bath salts* may be due to the powerful combination of a rapidly acting DA releasing agent and an incisive DA reuptake inhibitor acting at the human dopamine transporter.

Abstract 29

THE EFFECT OF NOTCH SIGNALING ON PERINEURIAL GLIA MIGRATION FROM THE SPINAL CORD

Laura Binari and Sarah Kucenas

Department of Biology, University of Virginia

Proper formation of peripheral motor nerves is vital for the passage of information out of the CNS to targets in the periphery. The three key cellular components of these nerves are motor axons, the Schwann cells that ensheath them, and perineurial glial cells, which form a protective barrier around the axon-Schwann cell complexes. During development, these cells migrate long distances in order to associate with one another to form the nascent motor nerve. However, what remains unclear are the mechanisms that control the migration of these nerve-associated glial cells during PNS assembly. The perineurial glial cells migrate from the CNS into the PNS via motor axon exit points to form the perineurium, which serves as the blood-nerve barrier in the PNS. In Drosophila, the Notch signaling pathway has been implicated in mediating peripheral glial migration and we sought to discover if this mechanism is conserved in vertebrates. To test this hypothesis, we pharmacologically inhibited Notch signaling during periods of perineurial migration. In conjunction with Notch responsive lines, we identified a developmental window in which Notch signaling, if inhibited, leads to a failure of perineurial glial migration from the CNS even though their membrane extensions are still capable of crossing into the periphery. Additionally, assays of nerve health demonstrated improper formation of the perineurium and a decrease in Schwann cells along the motor axons. These results indicate that Notch signaling is necessary to initiate migration of perineurial glia and that this abnormal migration affects formation of a healthy and differentiated nerve.

Abstract 30

CHARACTERIZATION OF A NOVEL ZEBRAFISH MUTANT WITH PERIPHERAL GLIAL DEFECTS: *FAILURE TO LAUNCH (FTL)* ^{VU268}

Jenna Eckardt and Sarah Kucenas

Department of Biology, University of Virginia

During development, neurons, Schwann cells and perineurial glia are mutually dependent on each other to form functional peripheral motor nerves. Each cell type originates from different precursor populations, but must migrate and differentiate in sync to meet and form the nerve fascicle. Most studies focus on axon-glial communication, however, we know very little about glial-glial interactions during nerve formation. Therefore our understanding of peripheral nerve assembly is incomplete. Our goal is to elucidate the role of Schwann cells and perineurial glia in nerve development and identify mechanisms by which they interact. To this end, a mutagenesis screen was performed in transgenic zebrafish and a mutant with a peripheral nerve defect was identified. This mutant, *failure to launch* (*ftl)*^{vu268}, was identified because the centrally-derived perineurial glia fail to migrate into the periphery. Initial characterization shows that Schwann cells are unperturbed early in development but show disruption later, after the perineurial glia fail to exit. Thus, we hypothesize that Schwann cell perturbation is a result of a lack a signal from the perineurial glia. Using a combination of molecular assays and live imaging, we will further characterize *ftl*^{vu268} to investigate spinal cord patterning and other aspects of perineurial development. Ultimately, we will map the locus that is mutated in this mutant line using bulk segregant analysis and chromosome walking. From these studies of the *ftl*^{vu268} mutant, we will identify a novel factor involved in PNS development and elucidate how this factor impacts the cell types in the developing nerve.

Abstract 31

THE ROLE OF MOTOR AXONS DURING PERIPHERAL GLIAL MIGRATION

Lauren Groskaufmanis and Sarah Kucenas

Department of Biology, University of Virginia

Development of the peripheral nervous system (PNS) is a complex process that involves the coordinated migration and differentiation of multiple cell types. Recent data demonstrate that successful development of motor nerves depends on synchronized interactions between axons, Schwann cells and perineurial glia. While some of the chemical signals that guide the progress of axonal growth cones have been identified, the signals that cue the migration and differentiation of glial cells are less well known. Originally thought to be solely guided by axonal cues, new research suggests that glial precursors may respond to cues from the periphery and migrate independently from axonal signals (Banerjee et all; 2011). In the absence of motor axons, Schwann cells can migrate to appropriate locations guided by signals from somatic muscle cells. Additionally, glial cells were observed migrating ahead of axonal growth cones, which suggests that glial cells play a previously unappreciated role in motor nerve development. To investigate the potential role of perineurial glia during nerve assembly, we are using the zebrafish *diwanka* (lh3) mutant that harbors a mutation in LH3, a myotomally expressed multi-functional enzyme. This enzyme modifies type XVIII collagen, used by axons for pathfinding. Abnormal axon development is observed but the effect on glial cells has not been shown. The *diwanka* fish, combined with molecular characterization and *in vivo* imaging, provides us a unique a tool for studying the hypothesis that like Schwann cells, perineurial glia are capable of pathfinding into the periphery in the absence of axons.

Abstract 32

NETRIN MEDIATES GLIAL CELL MIGRATION ACROSS THE CNS-PNS BOUNDARY

Taylor Huntington and Sarah Kucenas

Department of Biology, University of Virginia

The vertebrate nervous system is divided into two essential halves, the central and peripheral nervous systems (CNS and PNS, respectively), which are segregated via a structure known as the transition zone. This specialized boundary structure separates the distinct cell types and support elements of the CNS and PNS, regulates migration into the periphery, and defines the iterated motor exit and sensory entry points along the neural tube. Because of the many important roles of this structure, defects may contribute to several diseases of the nervous system. Recent studies have demonstrated that in order to form a functionally normal boundary, both Schwann cells and perineurial glia are necessary. However, few studies have researched how cell signaling between the cell types in the CNS and PNS assists in forming the transition zone. Netrins, secreted from the lateral floor plate where perinerual glial cells and oligodendrocyte precursors are located, are an attractive candidate to mediate these interactions. They are chemoattractant/repulsive guidance molecules that are known play a significant role in axon guidance and function in oligodendrocyte dispersal. Here we show that netrin 1b may play in integral role in the cell signaling that contributes to the formation of the CNS-PNS boundary through the use of time-lapse imaging, morpholino oligonucleotide (MO) injections, and molecular characterization. Fish with inhibited netrin 1b function show oligodendrocytes exiting via transition zones and significantly less perineurial migration.

Abstract 33

THE ORIGIN OF THE MAMMALIAN PERINEURIUM

Jessica E Kennett, Michael P Matisse and Sarah Kucenas

Department of Biology, University of Virginia

The development of motor nerves is dependent upon the coordinated assembly of its components, which include the motor axon, Schwann cells, the endoneurium, the perineurium and the epineurium. Motor nerve formation begins when a motor neuron projects its axon into the periphery via motor axon exit points (specialized CNS-PNS transition zones located at iterated positions along the anterior-posterior axis of vertebrates). Once in the periphery, Schwann cells, which are derived from the neural crest, wrap and myelinate the outgrowing motor axons. Outside of these axon-Schwann cell complexes, the perineurium ensheaths multiple myelinated axons into a fascicle, thus serving as a physical buffer from ionic flux, infection and toxins. Because of its location on the outside of the nerve, the perineurium plays a very important role in the maintenance of the peripheral nervous system. However, very little attention has been given to this important class of glia, namely its developmental origin. Recent work has shown that in *Drosophila* and zebrafish, perineurial cells originate as glia in the neural tube as opposed to the mesoderm, as has previously been suggested. More specifically, in zebrafish, *nkx2.2a*$^{+}$ cells from the lateral floorplate (p3 domain) of the spinal cord give rise to the perineurial cells that migrate into the periphery and ultimately form the mature perineurium. Here, we suggest that the same origin holds true for perineurial cells in the mouse. Using a transgenic mouse line in which lacz expression is driven by *nkx2.2*, we suggest that the perineurial cells of the mouse are similarly derived from the p3 domain of the spinal cord and migrate into the periphery to ensheath motor nerves and ultimately form the mature perineurium. Insight into the development of the peripheral nervous system will allow a better understanding of the etiology and progression of peripheral diseases states such as Charcot-Marie-Tooth.

Abstract 34

CHARACTERIZATION OF A NOVEL ZEBRAFISH MUTANT: *RUNAWAY (RAY)*^{VU267}

Rebecca Nash & Sarah Kucenas

Department of Biology, University of Virginia

Normal development of the nerves of the peripheral nervous system (PNS) is crucial to the survival and growth of an organism. Three major cell types, Schwann cells, perinuerial glia and motor axons, compose the peripheral nerves. These distinct cell types must coordinate their migration and differentiation to form a fully functioning and efficient PNS. While both perineurial glia and motor axons are born in and exit from the central nervous system (CNS), Schwann cells are derived from the neural crest and must migrate down the spinal cord, eventually myelinating the outgrowing axons. Perineurial glia provide an outer layer of protection by surrounding the Schwann cell-encased axons. Recent findings suggest that these three cell types must communicate with each other before, during and after development. However, the underlying mechanisms are largely unknown. Therefore, to gain further insight into the nature of nerve component communication during development, we are studying these three cell types in the context of a novel zebrafish mutant, *runaway* (*ray*^{vu267}). In *ray*^{vu267} mutants, perineurial glia exit the spinal cord erratically and ectopically. Preliminary characterization studies on the other motor nerve-associated cell types show that Schwann cells, while present, do not make myelin. Additionally, we observe fewer Schwann cells, suggesting that the *ray*^{vu267} mutation may specifically affect Schwann cells. Interestingly, initial characterization of motor axons does not show any defect. Follow-up phenotypic and genotypic studies will provide a better understanding of the *ray*^{vu267} mutation and subsequently of PNS developmental mechanisms.

Abstract 35

KROX20 DEFECTS IN THE PNS RESULT IN SCHWANN CELL DEATH AND ABNORMAL PERINEURIAL GLIAL DIFFERENTIATION.

Vatsal Patel and Sarah Kucenas

Department of Biology, University of Virginia

Normal development of the peripheral nervous system requires orchestrated interactions between axons and their associated glia. Historically, PNS studies focus almost exclusively on the interactions between only one class of glia, Schwann cells, and axons. In contrast, there is a nearly complete lack of understanding of the glial-glial interactions involving perineurial glia and Schwann cells during nerve formation. Previous studies show that the coordinated migration of axons, Schwann cells and perineurial cells is needed for normal peripheral nerve development. While this does shed some light on the interactions needed between Schwann cells and perineurial glia, the question still remains whether Schwann cell presence is required transiently or throughout development for normal perineurial differentiation. In this study, we are characterizing the requirement of Schwann cells for perineurial glial development. Specifically, we will be characterizing the *krox20/egr2b* zebrafish mutant, which causes Schwann cells to stall at the promyelinating stage. This mutation was specifically chosen to learn about the need for Schwann cells during late development. To characterize this mutant, double transgenic embryos were imaged and analyzed in terms of morphology and development of the peripheral nerves. Live imaging was supplemented with antibody labeling to further characterize differences in perineurial differentiation. Our preliminary data shows that interestingly, Schwann cells appear to die soon after 96 hpf. In addition, perineurial glia don't compact around axons. In the future, we will use transmission electron microscopy to analyze the ultrastructure of the nerves as this will reveal any subtle defects in the perineurium that may have been missed in more macroscopic imaging and characterization. Characterizing this mutation will result in a better understanding of when perineurial glia need Schwann cells and if it is a sustained requirement or only necessary at certain time points during nerve assembly.

Abstract 36

SCHWANN CELLS REQUIRE PERINEURIAL GLIA FOR THEIR DEVELOPMENT

Adithya Peruri and Sarah Kucenas

Department of Biology, University of Virginia

Efficiently functioning nerves require intimate and coordinated interactions between axons and the glia that ensheath them. Perineurial glia, which form the mature nerve perineurium, behave as a protective layer that encase axon-Schwann cell complexes. Although essential for nerve health, this class of glial cells has scarcely been investigated and their role in development is poorly understood. Previous findings demonstrate that in the absence of perineurial glia, motor axons ectopically exit from the central nervous system, suggesting that these glia play a role in axonal path finding out of the spinal cord. To further investigate the roles of perineurial glia during development, we are utilizing a zebrafish mutant known as monorail (*mol*), which harbors a mutation in the *foxa2* gene. In these mutant embryos, the floorplate fails to develop normally and the perineurial glia are affected. Using the mutant embryos as a tool, we hypothesize that because perineurial cells are closely juxtaposed to Schwann cells, aberrant perineurial development will affect Schwann cells. Through molecular characterization and *in vivo* imaging, we observe abnormal Schwann cell morphology and hallmarks of Schwann cell death. Additionally, Schwann cell migration is perturbed and perineurial glia fail to ensheath motor nerves. These data are consistent with the hypothesis that perineurial glia play a direct role in Schwann cell development and demonstrate that perineurial cells are essential mediators of PNS assembly.

Abstract 37

DEFECTIVE RETINAL DEPOLARIZING BIPOLAR CELLS (DBCS) IN REGULATORS OF G-PROTEIN SIGNALING (RGS) 7 AND 11 DOUBLE KNOCKOUT MICE

Hoon Shim[1] and Ching-Kang Chen[1]

[1]Department of Biochemistry and Molecular Biology, Virginia Commonwealth University School of Medicine, Richmond, VA 23220-0615

Purpose: The post-photoreceptoral loss of Gβ5S in the Gβ5 knockout (Gβ5-/-) mouse results in loss of the ERG b-wave. The protein levels of R7 subfamily of Regulators of G-protein Signaling (RGS): RGS6, RGS7, RGS9 and RGS11 are reduced in Gβ5-/- mice to near or below detection threshold. We have shown that RGS7 and RGS11 are co-localized on the dendritic tips of depolarizing bipolar cells (DBCs). In a RGS7 mutant mouse line termed SG7, which expresses a truncated RGS7 protein in reduced amounts, no differences were detected in ERG recordings when compared to wild-type (WT) controls. However, in RGS11 knockout (RGS11-/-) mice, the ERG b-wave was delayed. In the double mutant mouse line termed SG711 (SG7 and RGS11-/-), the ERG b-wave was further delayed. Since the SG7 mouse is a hypomorph, we sought to generate a true RGS7 null mouse (RGS7-/-) to investigate what the effect of a complete loss of RGS7 has on the ERG b-wave. With the true RGS7-/- on hand, we generated the complete RGS7/RGS11 double null mice and examined the ERG recordings from these animals.

Methods: The RGS7-/- mouse is generated by homologous recombination replacing exons 6-8 with a NEO cassette. The electroretinogram (ERG) is recorded under both scotopic and photopic conditions, bridged by a 10-minute light adaptation period at 30 cd/m^2 background intensity. The implicit times and amplitudes of both a- and b-waves are analyzed at various flash intensities. Protein levels are determined by Western blotting and protein localization is determined by immunohistochemistry. Ultrastructural abnormalities of the outer plexiform layer were examined by electron microscopy.

Results: The RGS7-/- mouse is smaller in size, but nonetheless viable and fertile. Knocking out RGS7 prolongs electroretinogram (ERG) b-wave implicit time in young animals immediately after eye opening. The b-wave defect disappears by two months of age. Expression levels of RGS6 and RGS11 are unchanged in RGS7-/- retina, but the level of the R7 RGS obligate partner G□5S is reduced. By characterizing a complete RGS7 and RGS11 double knockout (711dKO) mouse line, we found that G□5S expression in the retinal outer plexiform layer (OPL) is missing, as is the ERG b-wave. Ultrastructural defects similar to those found in G□5-/- mice are present in 711dKO mice.

Conclusions: The data indicates that RGS7 has an underappreciated role in ERG b-waves. In the RGS11-/- mouse, protein levels of Gβ5S do not change possibly due to the up-regulation of RGS7 to compensate for the loss of RGS11. However, in RGS7-/- mice, Gβ5S protein level decreases, while RGS6 and RGS11 levels do not change, which indicates that RGS7 may have a distinct role within the retina. While RGS6 alone sustains significant amount of G□5S expression in the retina, the absence of an ERG b-wave in G□5-/- mice is caused solely by the complete loss of RGS7 and RGS11.

Abstract 38

ETHANOL-INDUCED CELL FATE ALTERATIONS IN C. ELEGANS

Lindsay Kondo, Kalyann Kauv, Richard Raabe, Mia Bolling, Andrew Davies and Jill Bettinger

Virginia Commonwealth University, Department of Pharmacology and Toxicology

Fetal alcohol syndrome (FAS) is the leading preventable cause of mental retardation, but the molecular mechanisms underlying FAS are not well understood. We have taken a genetic approach to studying the effect of ethanol on a discreet cell fate decision occurring during embryogenesis in the nematode, *C. elegans*. AWC cells are a pair of olfactory neurons that allows *C.elegans* to discriminate between volatile attractive odorants in odor chemotaxis and odor discrimination behavioral assays. Early in development, AWC neurons make an activity dependent cell fate decision, and subsequently particular groups of G protein-coupled receptors are asymmetrically expressed in the two AWCs. A GFP tagged STR-2 allows us to monitor cell fate decisions between AWC neurons. SLO-1, a voltage-gated potassium channel is also expressed in these neurons, and activation of this channel can modify the AWC cell fate decision so that both AWCs adopt the same cell fate (which we identify as both cells expressing the GFP marker, or a 2 AWC^{ON} cell fate). Previous studies from our lab have shown that SLO-1 is a major molecular target of ethanol and mediates ethanol sensitivity. We tested if ethanol exposure during embryogenesis could cause defects in the AWC cell fate decision and found that ethanol exposure could alter the AWC cell fate, which requires the SLO-1 channel. Furthermore, by altering the lipid composition of the cell membrane, we can render this cell fate decision resistant to the effects of ethanol. To determine if this change in AWC cell fate has functional consequences, we are currently testing the ability of animals that had been exposed to ethanol during embryogenesis to perform in chemotaxis and odorant discrimination assays, which requires proper function of the AWCs. We predict that exposing embryonic worms to ethanol will cause functional behavioral changes in the animal due to altered AWC cell fate decisions.

Abstract 39

FOCUSING ON ACTIVATION IN THE BIPHASIC LOCOMOTOR RESPONSE TO ETHANOL

E. Gregory Hawkins, Jill Bettinger, Andrew Davies

Virginia Commonwealth University, Department of Pharmacology and Toxicology

Ethanol produces biphasic behavioral effects in which subjects are first hyperactive followed by a period of depression. The stimulating effects of ethanol are thought to result from acute exposure of low dose ethanol and have been demonstrated in invertebrates and mammals. The molecular mechanisms of ethanol's effects as a stimulant, versus that as a depressant, remain unclear. Our objective is to use genetic, behavioral, biochemical, and biophysical screens in *Caenorhabditis elegans* to determine the mechanism of ethanol-induced hyperactivity.

In a locomotor tracking assay control (N2) worms on food (OP50) are slower after 50 minutes compared to the 10-minute time point (150 ± 0.79μm/s at 10 minutes to 56.39 ± 12.51μm/s after 50 minutes). In contrast, in the presence of 300mM ethanol the N2 worms maintain their original speed over the 50-minute recording interval (132.18 ± 3.72μm/s at 10 minutes to 149.59 ± 13.99μm/s at 50 minutes). Using the same assay we also found that strains carrying mutations with defects in neuronal signaling actually increase their mean speed by treatment with ethanol. Three different alleles of *unc-13* (*e1091*, *e450* and *e51*) show a 100-300% increase in mean speed depending on allele and time. UNC-13 promotes the open state of the neuromuscular docking protein UNC-64 (syntaxin).

As a result, we propose that hyperactivity seen with ethanol treatment is related to proteins specific to neuronal signaling. The locomotor tracking assay will be used as the first screen to determine if/how ethanol modulates the activity of mutant worms with mutations affecting specific neuronal signaling proteins. Using a genetically encoded calcium indicator (such as G-CaMP) we propose to ascertain which neurons are involved in this signaling event. Our final aim is to use electrophysiology to measure the acute affect of ethanol at the neuromuscular junction and investigate how it is altered in our mutants. We believe these assays will allow us to determine which proteins are involved in the hyperactivity response to ethanol and how this response differs from the depressive response.

Abstract 40

MAGL INHIBITION: POTENTIAL TREATMENT FOR NICOTINE DEPENDENCE

Pretal Muldoon, Aron Lichtman, Ph.D., Imad Damaj, Ph.D.

Department of Pharmacology and Toxicology, Virginia Commonwealth University Richmond, VA, USA

Nicotine is the main addictive component of tobacco that plays a major role in dependence. Emerging evidence suggest that the endogenous endocannabinoid system may modulate these effects. Our lab has previously reported that increase in AEA, enhanced nicotine withdrawal and reward of nicotine and was CB1 mediated (Merritt et. al. 2008). However, 2-AG has yet to be studied in nicotine's effect. 2-AG is the most abundant endocannabinoid in the brain, required for cannabinoids synaptic transmission and exerts its action via CB1 receptors. To assess 2-AG's role in Nicotine Dependence (ND) we enhanced 2-AG levels via MAGL inhibition by JZL184. Nicotine reward in the mouse was evaluated in an unbiased conditioned place preference paradigm (CPP). Our results showed that degradation of MAGL dose-dependently decreased nicotine preference compared to nicotine control in our CPP paradigm. This blockade of Nicotine CPP is not CB1 mediated. Finally, we assessed JZL184's effect on another important aspect of ND, nicotine withdrawal. We wanted to assess the role 2-AG's neurotransmission in both physical and affective measures of nicotine withdrawal. JZL184 did not alter nicotine withdrawal induced anxiety-like behavior. However, JZL184 was able to dose-dependently block somatic signs and this effect was CB1 mediated. We then assessed JZL184's role in another affective measure the Nicotine Withdrawal Conditioned-Place Preference (CPA) model. Here, we took the lowest active dose of JZL184 (8mg/kg) and was able to completely block mecamylamine induced aversion. These results suggest that AEA and 2-AG have different roles in nicotine withdrawal and reward. MAGL inhibition is able to block both nicotine reward and physical somatic withdrawal signs. Interestingly, JZL184's effect in Nicotine CPP is not CB1 mediated whereas in physical somatic withdrawal signs they are.

Abstract 41

HIV-1 TAT AND OPIATE REDUCE NEURAL PROGENITOR CELLS: DOES THIS LEAD TO GENDER-SPECIFIC BEHAVIORAL EFFECTS?

Yun Kyung Hahn[1], Elizabeth M. Podhaizer[2], Sean P. Farris[2], Phu Vo[1], Sylvia Fitting[2], Michael F. Miles[2], Kurt F. Hauser[2], & Pamela E. Knapp[1,2]

[1]Depts. of Anatomy & Neurobiology, [2]Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA USA

HIV infected patients with a history of injection opiate abuse have higher incidences of acquired immunodeficiency syndrome (AIDS) and neurological dysfunction. The use of combined anti-retroviral therapy has significantly reduced the prevalence of mortality and progression to AIDS. Due to extended life expectancy, these patients are still at a great risk for HIV-associated neurological disorders and impairment in their later life. Neural progenitor cells (NPCs), which play critical roles in brain growth and repair after injury and insult, have been shown to be a potential target of HIV (Hahn et al, *J Neurochem*, 2010). Pediatric HIV patients whose glial populations are still developing are especially at risk for central nerve system (CNS) damage. Our previous reports suggest that HIV-1 Tat can directly cause pathology in neural progenitors and oligodendroglia (OLs) (Hauser et al, *Glia*, 2009). Thus, we have hypothesized that NPCs may be targets of HIV proteins ± opioid drugs of abuse. We examined effects of Tat and morphine on proliferation and lineage progression of NPCs *in vitro* and *in vivo*. *In vitro*, Tat and morphine independently altered the proliferation and population of $Sox2^+$ and $Olig2^+$ cells, but not $nestin^+$ cells, in the absence of cell death. The interactive effects of morphine and Tat varied depending on outcome measure and time of exposure. To investigate effects of Tat and morphine on NPCs *in vivo*, we used a mouse model in which HIV-1 $Tat_{1\text{-}86}$ is conditionally expressed in astroglia. *In vivo* results in neonatal striata were similar to those in cultures. We next extended the experiments into adult mice, inducing Tat expression for 3 mo and also examining the effect of gender. $Sox2^+$ and $Olig2^+$ cells showed proliferation/population changes in both genders. Intriguingly, when we did behavioral tests (rotarod, grip strength, locomotor activity, light-dark box), males showed more Tat-induced impairment. Gender differences were seen in the proportion of $3\text{-nitrotyrosine}^+/Iba^+$ cells, with Tat^+ males showing greater microglial activation. Tat^+ males also showed greater reduction in the proportion of $NeuN^+$ cells in the striatum. Both the enhanced microglia reactivity and reduced proportions of $NeuN^+$ cells in males may help to explain gender-specific behavioral outcomes. Overall, our findings show that CNS progenitors are selectively vulnerable to individual or combined effects of HIV-1 Tat and opiates, in a manner that shows temporal and lineage-specificity. Changes in progenitor dynamics will likely alter the balance of cell populations in the CNS. We speculate that such changes may contribute to behavioral abnormalities in the Tat^+ mice. (DA024461, NS069216)

THE DEGREE OF RETINAL CONVERGENCE ONTO INTERNEURONS IN THE DORSAL LATERAL GENICULATE NUCLEUS OF THE MOUSE IS MAINTAINED DURING POSTNATAL DEVELOPMENT.

Tania A. Seabrook, Emily K. Dilger, Rana N. El-Danaf, Thomas E. Krahe, William Guido

Department of Anatomy & Neurobiology, Virginia Commonwealth University, Richmond, VA

Two general classes of neuron are present within the dorsal lateral geniculate nucleus (dLGN), relay cells and interneurons. While both receive direct input from the retina, studies on the development of the retinogeniculate pathway have focused largely on the connections between retinal ganglion cells and relay cells. Little is known about the pattern of retinal convergence onto interneurons and whether they, like relay cells, undergo a period of pruning during early postnatal life. To address this we conducted *in vitro* whole-cell recordings from acute thalamic slices of GAD67-GFP mice, in which GFP is expressed in dLGN interneurons. Since interneurons comprise a small fraction of neurons in dLGN, GFP expression allowed us to readily identify and target them during *in vitro* recordings. To estimate the degree of retinal convergence onto interneurons, we prepared a slice in which the retinal connections and intrinsic circuitry of dLGN were maintained. Electrical stimulation of optic tract was used to evoke excitatory postsynaptic potentials (EPSPs) in interneurons. Across all ages tested a progressive increase in stimulus intensity led to a graded increase in EPSP amplitude. Estimates of retinal convergence derived from EPSP by stimulus intensity plots revealed that interneurons received on average about 8 retinal inputs. Even at late postnatal ages interneurons received as many as 10-13 inputs. Biocytin-labeled interneurons filled during recordings were imaged using multi-photon laser scanning microscopy to obtain 3-D reconstructions of their dendritic processes. Retinal terminals from both eyes were labeled with cholera toxin subunit B (CTB) conjugated to Alexa dyes in order to visualize retinal contacts on interneurons. The dendrites of interneurons were quite long, spanned large regions of dLGN, and readily crossed eye-specific borders. Retinal contacts were widely distributed on the soma and both proximal and distal regions of dendrites. Thus unlike relay cells, interneurons appear to receive input from several retinal ganglion cells with contacts scattered throughout their dendritic trees. Since high rates of convergence were apparent even at late postnatal ages it seems that interneurons are perhaps immune to the influence of early spontaneous retinal activity, an event that figures prominently in the segregation of eye specific modules and pruning of retinal ganglion cell axons.

Abstract 43

EPHRIN-B2 IS NECESSARY FOR ACCURATE TOPOGRAPHY BUT NOT REQUIRED FOR PATTERN FORMATION OF LATERAL SUPERIOR OLIVARY INPUTS TO THE INFERIOR COLLICULUS PRIOR TO HEARING ONSET.

Matthew M. Wallace, Devon R. Cowan, Mark L. Gabriele

Dept. of Biology, James Madison Univ, Harrisonburg, VA, USA

Graded and modular expressions of Eph-ephrins are known to provide positional information for the formation of topographic maps and patterning in the developing nervous system. Utilizing immunocytochemistry in control animals and X-Gal staining approaches in *lacZ* mutants we have shown that ephrin-B2 expression patterns exhibit a continuous gradient across the tonotopic axis of the central nucleus of the inferior colliculus (CNIC), whereas patterns are discontinuous and modular in the lateral cortex of the IC (LCIC). As converging layered and modular inputs target specific domains within the developing CNIC and LCIC respectively, ephrin-B2 patterns are distinct prior to their downregulation as experience ensues (functional onset of hearing in mouse, P11/12). The present study explores the involvement of ephrin-B2 signaling in the development of projections to the CNIC and LCIC arising from the lateral superior olivary nuclei (LSO). Fluorescent tracing methods in early postnatal fixed tissue preparations were used to compare axonal targeting and establishment of LSO layers/modules in wild-type and ephrin-B2$^{lacZ/+}$ mice (ephrin-B2$^{lacZ/+}$ incapable of reverse signaling; ephrin-B2$^{lacZ/lacZ}$ perinatally lethal). While axonal trajectories of pioneer LSO fibers in ephrin-B2 mutants are somewhat aberrant at birth, both the ipsilateral and contralateral projections form discernible layers and modules in the developing CNIC and LCIC. In contrast, absence of a strict topography in LSO-IC projections in the mutant suggests ephrin-B2 reverse signaling is required for accurate axonal targeting.

Abstract 44

X-GAL STAINING OF *LACZ* EPHRIN-B2 AND EPRHIN-B3 MUTANT MICE IN THE AUDITORY MIDBRAIN PRIOR TO HEARING ONSET

Caitlyn A. Klotz, J. Aaron Harris, Matthew M. Wallace, and Mark L. Gabriele

Dept. of Biology, James Madison Univ, Harrisonburg, VA, USA

A family of receptor tyrosine kinases, the Eph-ephrins, are thought to provide signaling cues necessary for the establishment of highly organized circuits in the ascending auditory system. Of particular interest to our laboratory is their involvement in the formation of patterned projections arising from multiple brainstem sources and terminating in the auditory midbrain, or inferior colliculus (IC). The IC is composed of three subdivisions: central nucleus (CNIC), lateral cortex (LCIC), and dorsal cortex (DCIC). The present study examines the relative expression patterns of two members of this family (ephrin-B2, -B3) in the developing IC leading up to the onset of experience (postnatal day 12, P12; mouse). Previous findings in our laboratory utilizing immunocytochemical approaches reveal that these proteins are indeed present during this developmental period and exhibit graded and modular expression patterns that mimic the known topography and compartmentalization of the CNIC and LCIC. Here we confirm these findings using X-gal staining methods in ephrin-B2, -B3 *lacZ* mutants. The results indicate a gradient of ephrin-B2 from high frequency to low frequency across the tonotopic axis of the CNIC. In addition, prominent ephrin-B2 modules are evident throughout the LCIC. In contrast, the CNIC is devoid of ephrin-B3 protein, while LCIC expression appears to complement that of ephrin-B2. Interestingly, a downregulation of both proteins is observed that is coincident with the functional onset of hearing. These findings confirm previous immunocytochemistry data and provide a framework for understanding *in vivo* projection development in mutant models where Eph-ephrin signaling is disrupted.

Abstract 45

DUAL ROLES FOR AGGRECAN IN CELL-TYPE SPECIFIC AXONAL TARGETING OF THE DORSAL LATERAL GENICULATE NUCLEUS

Brooks, J., Su, J., and Fox, M.A.

Department of Anatomy and Neurobiology Virginia Commonwealth University Medical Campus, Richmond, VA

Visual system development requires the precise formation of synapses onto relay neurons in the lateral geniculate nucleus (LGN). Image-forming classes of retinal ganglion cells (RGCs) innervate relay neurons in the dorsal LGN (dLGN), whereas, axons from non-image forming classes target more ventral regions of the LGN: the intergeniculate leaflet (IGL) and ventral LGN (vLGN). Relay neurons in the dLGN also receive input from layer VI cortical neurons, but these corticogeniculate circuits pause outside of the LGN and do not invade dLGN until retinogeniculate circuits have matured. The cellular and molecular mechanisms regulating spatial and temporal axonal targeting of the LGN by these classes of retinal and cortical neurons are largely unknown. To address both of these issues, we applied microarray and immunohistochemical screens to identify guidance and targeting cues whose expression was restricted or enriched in one LGN subnucleus and whose expression correlated with timing of retinogeniculate or corticogeniculate innervation. We discovered that aggrecan, a chondrotin sulfate proteoglycan (CSPG) that repels growing axons, was enriched in dLGN at birth. Subsequently we discovered that aggrecan was degraded by a family of extracellular enzymes (i.e. the *a* *d*isintegrin *a*nd *m*etalloproteinase with *t*hromobo*s*pondin motifs [ADAMTS] family) enriched in dLGN after retinogeniculate targeting. The upregulation of ADAMTS and degradation of aggrecan coincided with the invasion of corticogeniculate axons. We, therefore, hypothesize dual roles for aggrecan in dLGN circuit development. At early ages, aggrecan may be necessary for class-specific targeting of retinal projections in the dLGN, and later in development aggrecan may be necessary for preventing premature innervation of dLGN by corticogeniculate axons. Here we use transgenic and mutant mice and *in vitro* assays to test the role of aggrecan in the spatial and temporal regulation of dLGN circuit formation.

TARGET-DERIVED MATRICRYPTINS ORGANIZE CEREBELLAR SYNAPSE FORMATION THROUGH α3β1 INTEGRINS

Jianmin Su[1], Renee S. Stenbjorn[1], Kaiwen Su[1], Sylvie Ricard-Blum[2], Taina Pihlajaniemi[3,4,5], Michael A. Fox[1*]

[1]Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA

[2]Institut de Biologie et de Chimie des Protéines , UMR 5086 CNRS, University Lyon 1, France

[3]Oulu Center for Cell-Matrix Research, [4]Biocenter Oulu, [5]Department of Medical Biochemistry and Molecular Biology, 90014 University of Oulu, Finland

Trans-synaptic organizing cues must be passed between synaptic partners for synapses to properly form. Much of our understanding of this process stems from studies at the neuromuscular junction (NMJ) where target-derived growth factors, extracellular matrix (ECM) molecules, and matricryptins (proteolytically released fragments of ECM molecules) are all essential for the formation and maintenance of motor nerve terminals. While growth factors and ECM molecules also contribute to the formation of brain synapses, it remains unclear whether similar roles exist for matricryptins in the mammalian brain. Here, we report that endostatin, a collagen XVIII-derived matricryptin, is generated by cerebellar Purkinje cells and is necessary for the assembly of climbing fiber terminals onto these neurons. Application of endostatin onto cultured inferior olivary neurons induces climbing fiber terminal differentiation by signaling through α3β1 integrins. Taken together, these studies reveal novel roles for both matricryptins and integrins in the assembly of brain synapses.

THE USE OF *IN SITU* HYBRIDIZATION TO CHARACTERIZE THE MOLECULAR COMPOSITION OF HYPOTHALAMIC THERMOREGULATORY NEURONS IN THE HYPOTHALAMUS OF THE RAT

Andrew Moore, Monika Stanciauskas and John D. Griffin

Department of Biology, The College of William and Mary, Williamsburg, Virginia 23187-8795, USA

The thermoregulatory pathway in the hypothalamus focuses largely on two types of neurons: temperature-sensitive neurons and temperature-insensitive neurons. As their names imply, the primary difference between these two neurons is how they react to changes in temperature. Currently, the only way to differentiate between the two types of neurons is to isolate them and see how they react to changes in temperature. The most common method for researching these neurons involves intracellularly recording from the cells, which allow researchers to infer regulatory pathways based on how the neurons respond to temperature changes and various chemicals. This method, however, provides no means for studying the genetic expression of the cells. In this new method we will be able to determine the specific molecular composition of temperature-sensitive and temperature-insensitive neurons. To do this, we combine intracellular recordings with *in situ* hybridization (ISH) to provide us with information concerning the differential gene expression in the two types of neurons. Our current study focuses largely on expression of the VGLUT2 gene in temperature-sensitive versus temperature-insensitive neurons. By staining the cells that we record from intracellularly with Lucifer yellow, we can utilize ISH to determine whether those neurons are glutamatergic or not. This technique allows researchers to quickly and easily study the differential gene expression seen in thermoregulatory neurons in the hypothalamus. Ultimately, we hope to use this technique to find a molecular difference between the two types of neurons in order to more easily differentiate the two for further studies. This research was supported by grants from the NIH (NS053794, NS065461 & NS053794-S1) and in part by a Howard Hughes Medical Institute grant through the Undergraduate Science Program to the College of William and Mary.

Abstract 48

THE EFFECTS OF THE ALPHA-2 ADRENORECEPTOR AGONIST CLONIDINE ON THE ACTIVITY OF THERMALLY CLASSIFIED NEURONS IN THE ANTERIOR HYPOTHALAMUS OF THE RAT

Reinaldo Perez, Andrew Moore, and John D. Griffin

Department of Biology, The College of William and Mary, Williamsburg, Virginia 23187-8795, USA

In response to infection, noradrenergic vagal afferents to the hypothalamus may be responsible for a rapid biphasic shift in thermoregulatory control. An initial hypothermic shift may result from the activation of alpha-2 adrenoreceptors, which is followed by an enduring hyperthermic response that is initiated by activation of alpha-1 andrenoreceptors and prolonged by the presence of prostaglandin E2 (PGE2). While previous studies from our lab have characterized the effects of PGE2 (Ranels & Griffin, 2003) and the alpha-1 agonist Cirazoline (Imbery et al., 2007), this study recorded the in vitro single-unit activity of thermally classified anterior hypothalamic neurons in response to the alpha-2 adrenoreceptor agonist Clonidine (100 μM). Based on their responses to temperature, warm sensitive neurons ($m > 0.8$ impulses.s^{-1}.$°C^{-1}$) showed significant increases in firing rate while temperature insensitive neurons showed significant decreases in firing rate. These changes would result in a hypothermic response according to Hammel's model, demonstrating the role of this adrenoreceptor subtype in temperature regulation. This research was supported by grants from the NIH (NS053794, NS065461 & NS053794-S1) and in part by a Howard Hughes Medical Institute grant through the Undergraduate Science Program to the College of William and Mary.

AXONAL DIEBACK FOLLOWING TRAUMATIC BRAIN INJURY RESULTS FROM LOCAL MACROPHAGE ACTIVATION RATHER THAN RETINAL GANGLION CELL DEATH

Jiaqiong Wang, John Povlishock

Virginia Commonwealth University, Richmond, VA, USA

PURPOSE: Traumatic brain injury (TBI) is a major health problem with most of its morbidity associated with traumatic axonal injury (TAI). Previously, we have developed a model of TAI in the optic nerve, wherein the TAI evolved rapidly with dramatic anterograde and retrograde axonal dieback. Given this dramatic damage and its rapid progression, we questioned whether this was the result of local inflammation and/or concomitant cell death within the retina.

METHODS: YFP-16 transgenic mice were subjected to mild central fluid percussion injury at 1.40 ± 0.05atm and allowed to survive from 24h to 28d post TBI. Optic nerve frozen sections were reacted with antibodies targeting astrocytes (GFAP) and microglia/macrophages (Iba1). Retina frozen sections were immunolabeled with antibodies to the cleaved caspase-3 as well as TUNEL approaches interfaced with electron microscopy.

RESULTS: Both reactive astrocytes and numerous activated microglia=macrophages were recognized in the optic nerve at 24h and 48h post TBI, with many of the activated microglia/macrophage lying adjacent to swollen axonal segments in the process of dieback. YFP positive cells in the retinal ganglion cell layer revealed no overt loss at 2d, 7d and 14d post TBI. Further, they were not labeled by the cleaved caspase-3 antibody. Overall, the optic nerve showed improved structural detail and reorganization at 28d post TBI in comparison to that seen at 24h and 48h post TBI.

CONCLUSION: Collectively, our results suggest that astrocyte and microglia/macrophage activation are associated with the axonal dieback identified in the pathogenesis of TAI within the optic nerve. The absence of concomitant retinal degeneration and/or ganglion cell death argues that they are not directly involved within the dieback process. The implications of these findings for our understanding of TAI will be discussed, together with a consideration of their relevance to other optic nerve injury models.

(Supported by HD055813 and NS047463)

WE ARE EXTREMELY GRATEFUL FOR THE GENEROUS DONATIONS SUPPORTING THIS SYMPOSIUM FROM:

VCU School of Medicine Dean's Office
VCU Life Sciences Department
VCU Department of Anatomy and Neurobiology
VCU Interdisciplinary Neuroscience Graduate Program
VCU Department of Pharmacology and Toxicology
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